The Role of Airway Mucus in Pulmonary Toxicology

James M. Samet[1,2] and Pi-Wan Cheng[3,4]

[1]Center for Environmental Medicine and Lung Biology, [2]Curriculum in Toxicology, [3]Department of Pediatrics, and [4]Department of Biochemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Airway mucus is a complex airway secretion whose primary function as part of the mucociliary transport mechanism is to to serve as renewable and transportable barrier against inhaled particulates and toxic agents. The rheologic properties necessary for this function are imparted by glycoproteins, or mucins. Some respiratory disease states, e.g., asthma, cystic fibrosis, and bronchitis, are characterized by quantitative and qualitative changes in mucus biosynthesis that contribute to pulmonary pathology. Similar alterations in various aspects of mucin biochemistry and biophysics, leading to mucus hypersecretion and altered mucus rheology, result from inhalation of certain air pollutants, such as ozone, sulfur dioxide, nitrogen dioxide, and cigarette smoke. The consequences of these pollutant-induced alterations in mucus biology are discussed in the context of pulmonary pathophysiology and toxicology. — Environ Health Perspect 102(Suppl 2):89–103 (1994).

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Introduction

The lung is a unique organ in it consists of a very large epithelial surface that is continuously exposed to the outside world. Unlike a similarly exposed epithelial surface, the skin, the lung cannot make use of multiple layers of relatively impermeable cells as a barrier to harmful substances in the outside environment. This is because the function of the lung, i.e., gas exchange, demands that there be a minimal thickness of gas-permeable cell membranes between the airspace and the blood. Instead, the lung uses specialized secretions produced by the airways to provide a renewable and transportable protective layer to interact with, neutralize and remove inhaled toxic materials. Mucus is the principal airway secretion with this function.

Airway mucus is a viscous solution with defined physical and chemical properties that enable it to be transported out of the lungs by the ciliary motion of the ciliated cells lining the airways. Thus airway mucus is part of the mucociliary clearance mechanism, also known as the mucociliary escalator, that continuously sweeps trapped or neutralized inhaled materials out of the airways. This system also provides a vehicle for the removal of alveolar macrophages, the principal resident phagocytic cell in the lung, whose function is to ingest microorganisms and other particulates that reach the alveolar space. Alterations in mucus properties can occur as a result of certain disease states and exposure to inhaled toxic compounds. As will be discussed, these alterations can cause impaired pulmonary clearance and a number of pathologic conditions leading to, and resulting from, excessive mucus secretion.

The focus of this report is the role of mucin in the pulmonary toxicology of air pollutants. Included are sections describing current concepts in mucin biology, i.e., its biochemistry, biophysics, and histochemical properties, as well as its function in normal and pathophysiology. This is followed by a review of effects on mucin biology that are relevant to the pulmonary toxicology of inhaled pollutants.

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Address correspondence to Dr. James M. Samet, Section on Pulmonary Medicine and Critical Care, Bowman Gray School of Medicine, Winston-Salem, NC 27157-1054. Telephone (919) 716-6826. Fax (919) 716-2273

Mucus Composition

The classic model of the airway mucociliary system depicts mucus as a layer of highly viscoelastic luminal secretions floating on a layer of fluid of low viscosity (the sol or periciliary fluid layer). Mucus from healthy subjects is difficult to obtain because, in the absence of trauma or disease, very little is produced by the lung (*1,2*). Furthermore, even under carefully controlled conditions normal mucus contains significant quantities of cellular debris and airborne materials that confound analyses. Largely due to the lack of sufficient uncontaminated material available for study, the composition of normal human mucus is still uncertain (*1,3*). Analysis of sputum obtained from diseased subjects, normal secretions induced with hypertonic saline and recovered secretions from laryngectomized patients has yielded the consensus that mucus is 95% water, 2% glycoproteins, 1% proteins, 1% lipids, and 1% inorganic salts (*1–4*).

In contrast to normal airway secretions, mucus produced in the airways of patients with respiratory disease contains significant amounts of serum proteins. These proteins are believed to be responsible for alterations in the rheology of airway secretions characteristic of some pathologic conditions such as bronchitis and bronchiectasis (*3*). Some studies have suggested that at least part of the lipid in intestinal mucus is in the form of fatty acids covalently attached to glycoproteins (*5,6*). Airway glycoproteins, however, appear to contain small amounts of fatty acids that are noncovalently associated with hydrophobic regions of these molecules (*7*). For an excellent discussion of nonmucin proteins, antioxidants and other organic constituents of airway mucus, the reader is referred to a review by Hatch (*8*).

Water Content

Regulation of water content is crucial to maintaining viscoelasticity that is optimal for the transfer of momentum from the beating cilia of the airway epithelium to the mucus blanket of the mucociliary clearance system (*1*). A considerable fraction of the water in mucus is bound to the macromolecules or physically trapped inside the interstices formed by dissolved glycoproteins (*3*). The amount of water in mucus is determined by the availability of water in the airway, which is tightly linked to the

movement of the ions that make up the inorganic salts found in mucus.

The source of mucus water, and for that matter all airway water, is vascular transudation from the blood (*3*). This transudation results, in part, from the difference in hydrostatic pressure between the vascular bed and the airspace. However, the main mechanism for moving water into the airway is provided by an osmotic gradient established by the vectorial transport of ions into the lumen carried out by the airway epithelium. The tracheobronchial epithelium secretes Cl^- ions and absorbs Na^+ ions. Under normal conditions, the amount of Cl^- ion secreted exceeds the amount of Na^+ ion taken in, establishing an ionic gradient for net water secretion into the airway (*9*). Water lost by evaporation during the humidification of inspired air also increases the osmotic gradient and, thus, the water movement into the airway lumen (*10*). The ability to maintain the proper ionic composition of airway secretions is essential to maintaining normal mucociliary clearance. A defect in the mechanisms that regulate ion balance in the airway lumen is the central pathogenic feature of cystic fibrosis (*9*).

Mucus Rheology

In terms of both normal and pathophysiology, viscosity and elasticity are the most important physical properties of mucus, enabling it to be transported by ciliary movement (*11*). The principal determinant of the viscoelastic properties of mucus are the mucous glycoproteins, or mucins. Mucins are structural and secretory products of all secretory epithelia, including the airway epithelium. These glycoproteins are normally found as a dissolved, entangled network in mucus. When dissolved in purified form, mucins have been shown to form a gel (*12*) and recreate the rheologic properties of mucus (*1*). It is for these reasons that mucins and the factors that affect their structure and expression in health and disease have been extensively studied. Many modern methodological approaches to the study of the physicomechanical properties of this complex material have been developed, as recently reviewed (*13*). They range from models that seek to mathematically describe and predict the behavior of mucins in solution (*14*), to two-phase gas-liquid flow (*15*), magnetic rheometry (*16*), and sinusoidal oscillation methods (*17*).

Biochemical Analysis

Mucins are complex and heterogeneous molecules. They have been classified as acidic and neutral species, depending on their content of acidic groups, e.g., sulfate or sialic acid. Based on their sensitivity to neuraminidase, mucins have been further grouped into sialic acid-containing and sialic acid-free species (*18*). Acidic and neutral glycoproteins can be distinguished histochemicaly with Alcian blue-periodic acid Schiff stain, with which acidic glycoproteins stain purple and neutral ones appear magenta (*19*). This classification scheme is useful in evaluating mucin histochemical changes that occur in a variety of disease processes. However, it is noted that even "neutral" mucins contain sialic acid and sulfate when analyzed chemically.

Mucins are polydisperse and highly glycosylated molecules with molecular weights varying from a few hundred thousand to well over a million daltons (*20*). Mucin polydispersity may be the result of variation in peptide size (*21*). The classic structural representation of mucin is that of the "bottle brush," which depicts hundreds of carbohydrate chains attached to a peptide core (*22*). Typical methodology for the analysis of mucin (*23*) includes extraction with 8 M urea (*24,25*) or a chaotropic agent such as 6 M guanidinium chloride in the presence of endogenous protease inhibitors. After low-speed centrifugation to remove insoluble material, mucins are isolated in the void volume of an agarose gel column and further purified by CsCl density gradient centrifugation according to their density. Characterization of mucin glycoproteins is accomplished by agarose-polyacrylamide gel electrophoresis (*26,27*) with silver staining and analytical ultracentrifugation (*11,28*). Electron microscopy is also widely used for the analysis of the size and architecture of mucin glycoproteins. This technique has revealed that these molecules are flexible random coils with lengths ranging from 0.2 to 5 μm (*29–31*), although variations between studies exist, apparently due to differences in sample preparation (*32*). Sample handling and preparation has a significant effect on the integrity of mucins analyzed by other methodologies as well. To isolate intact mucins, the once standard high-shear extraction methods and disulfide bond-breaking reagents such as dithiothreitol are now avoided (*11*).

Mucin Peptides

Until recently, mucins were thought to have relatively conserved amino acid sequences (*1*). However, recent evidence obtained using methodologies that preserve the protein core of these glycoproteins, as well as new gene sequence information, suggests that there are multiple and polydisperse peptides, or apomucins (*33,34*). Protease-sensitive, sparsely glycosylated regions are found at both or one end of the peptide and are rich in aspartate and cysteine (approximately 13 to 16 per region). These cysteine residues form disulfide bonds within and between peptides and appear to dictate the folding of the assembled mucins (*1*). The glycosylated regions of mucin are protease-resistant segments rich in serine and threonine residues linked to carbohydrate chains (*22*).

Carbohydrate Content

Approximately 80% of the weight of the mucin molecule is carbohydrate (*33*), and these covalently attached sugars confer mucins with most of their heterogeneity and structural complexity (*22*). These carbohydrates are arranged in chains, called mucin type-glycans that include fucose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine, and *N*-acetylneuraminic acid. Mucin type-glycans are linked to the peptide at a serine or threonine residue by *N*-acetylgalactosamine that is alkali-labile (*22,35*). Reductive elimination frees the mucin type-glycan from the peptide and produces chains that range in size from 1 (*N*-acetylgalactosamine) to 20 carbohydrate residues (*36*). In addition, mucin oligosaccharide structures vary greatly with regard to anomeric configuration and linkages. For example, galactose can link to an adjacent sugar, either galactose, *N*-acetylglucosamine, or *N*-acetylgalactosamine, in α or β anomeric forms and in 1–3, 1–4, or 1–6 linkages. Mucin glycans can be classified into three groups of increasing acidity: neutral, sialylated, and sulfated (*22,36–38*). To date, over 100 mucin oligosaccharides have been identified, including 81 from one subject alone (*22,37,39–41*).

Functional Histology

Glycoprotein-Secreting Cell Types

The lungs of a typical healthy adult secrete mucus at a rate of 0.1 to 0.3 ml/kg body weight per day. Airway mucus is produced and secreted by several different cell types. Perhaps the most specialized cell for this task is the goblet cell, found mainly scattered among ciliated cells in the trachea and, in fewer numbers, in the bronchi (*42*). Goblet cells are the only example of a unicellular gland in mammals, their main secretory product being mucin. As its name implies, this is a vessel-shaped cell with an expanded apical end that is filled with

secretory granules. These granules are secreted from the cell as the membrane surrounding individual granules or coalesced groups of granules fuses with the plasma membrane, spilling their contents out of the cell. Under certain stimuli, goblet cells are capable of secreting all of their granules at once (*10,43*).

Clara cells are the most prevalent cell type found in the small airways and are capable of differentiating into other cell types of airway epithelium such as goblet cells (*44*). Clara cells are known contributors to the sol, or periciliary, fluid layer and are also the principal site of xenobiotic metabolism in the lung (*10*). Serous cells are another type of glycoprotein-secreting cell. These cells mainly produce neutral glycoproteins which are found packaged in distinct, electron-dense granules (*45*). Interestingly, serous cells appear to secrete *N*-glycosylated glycoproteins (*46*), a type of glycoprotein related to, but distinct from, the *O*-glycans. Serous cells are known to be capable of responding to inhaled irritants by undergoing a form of metaplasia to become goblet cells (*44,47*).

Serous cells are arranged within the terminal portion of the acini (an acinus is a rounded exocrine secretory unit) of submucosal glands (*43*). The approximately 5000 submucosal glands found under the airway epithelium between the trachea and the subsegmental bronchi are the major source of mucus in the normal lung (*10*). The proximal terminus of the submucosal glands are lined by mucus-secreting cells. These cells are packed with confluent, electron-lucent mucigen granules (*43*). Mucus acini in submucosal glands secrete mainly glycoprotein, while serous acini also secrete antibacterial compounds and antiproteases (*48*). The size and number of submucosal glands increases when the airway is exposed to environmental irritants such as tobacco smoke (*43*).

Morphometric studies on airway goblet cells and gland cells have shown that mucus is secreted from these cells as droplets 1 to 2 μm in diameter (*49*). The droplets are believed to be made up of concentrated glycoproteins, which in a matter of seconds absorb several hundred fold their weight and volume in water drawn from the periciliary fluid (*50*). As the glycoprotein droplets swell, they begin to form plaques or "rafts" of mucus that move over the periciliary fluid. As the diameter of the airways increases, these rafts coalesce into larger islands of mucus, to eventually form a sheet of mucus (*51*).

All secreted mucus is eventually transported out of the airways to the top of the trachea, where it is swallowed. This process is referred to as mucociliary function. It is estimated that 10 ml of mucus reaches the top of the trachea in a healthy adult every day (*52*). The mucus layer over the airway epithelium is approximately 5 to 10 μm thick and flows towards the trachea at a rate of 4.2 to 7 mm/min (*53*). Mucus flow at the trachea is between 7 and 25 mm/min (*10*). Factors affecting the rate of mucus flow include the thickness of the periciliary fluid layer and the relative humidity of inspired air, ciliary beat frequency, gravity, and airflow. The contribution of these factors to the mucus flow will be discussed next.

Periciliary Fluid

Surprisingly little is known about the periciliary fluid layer. It is believed to be an epithelial cell exudate (*54*), 6 μm in depth (*10*), having low viscosity and an ionic content that is tightly maintained by the movement of sodium and chloride by the airway cells (*55*). The periciliary layer in the distal airways and respiratory bronchioles probably contains some surfactant and other alveolar components (*56*). A phospholipid layer appears to exist between the periciliary fluid layer and the mucus sheet, and may lower the surface tension between the two layers (*57*). The thickness of the periciliary fluid layer provides a low viscosity medium between the cell surface and the mucus layer in which the cilia can beat and propel the mucus blanket (*44*).

The relative humidity of inspired air determines the rate of evaporation of fluid from the mucus sheet. This effect is most evident in the upper airways and the trachea, where incomplete humidification of inspired air is more likely (*58*). The effect of airway evaporative loss on the depth of the periciliary fluid layer is not known (*10*).

Mucociliary Transport

Another key determinant of the rate of mucus flow is the ciliary beat frequency. The movement of secreted mucus is carried out by ciliated epithelial cells, the predominant cell type in the airways. Ciliated cells in the airway are arranged in close juxtaposition, forming a field of cilia, interrupted by islands of goblet cells and mucus gland openings (*42*). The cilia beat in a synchronized, wavelike manner to propel the mucus towards the trachea. Each ciliated cell has 200 to 300 uniformly spaced cilia about 6 μm in length (*10*). The cilia project into the periciliary fluid, with only their tips embedded in the mucus layer. Each cilium is 0.25 μm in diameter and is composed of an axoneme surrounded by a special process of the plasma membrane called the ciliary membrane (*42,59*). The axoneme itself is made up of nine interconnected doublets of microtubules surrounding a pair of central microtubules. The axoneme is anchored via the basal body to the network of microtubules of the cell cortex. The basal body is composed of nine interconnected triplet microtubules. Each microtubule is made of protofilaments of tubulin, a self-assembling cellular protein. The microtubule doublets are linked to each other via arms of dynein, a protein with ATP-ase activity (*43*). The length of the cilium of the respiratory epithelium is a compromise between the bioenergetic advantages offered by a longer dynein arm and the required stiffness imparted by minimal length (*56*).

During ciliary movement, the axoneme microtubule doublets slide past each other. The binding of ATP to dynein releases and shortens the arm linking the doublets. As the ATP is hydrolyzed, the linkage between the doublets reforms at a more proximal site (*43*). This cycle of attachment and reattachment of dynein arms between microtubule doublets is repeated many times during each ciliary beat. The beating of groups of neighboring cilia is synchronized into "metachronal waves" as a result of a similarity in viscous forces experienced by the cilia at a given location (*44*). A similar self-regulating mechanism is responsible for enlisting the number of beating cilia necessary to keep mucus flowing at a steady rate. A given patch of mucus is propelled by a group of many cilia beating in a coordinated fashion. As the movement of the cilium is slowed down by the inertia of the slower-moving mucus blanket, other cilia catch up with it and transfer their energy to the mucus as well. The net effect is that the slower the mucus blanket moves over a given area of the epithelium, the greater the number of synchronized cilia propelling it (*44*).

Due to its high glycoprotein content, mucus behaves as a non-Newtonian fluid, with elastic properties that cause it to temporarily absorb energy by changing its shape (*56*). The amount of time elapsed between the deformation of mucus and its recoil to its original shape is refered to as the relaxation time, which has been measured to be approximately 30 sec for mucus (*60*). Efficient transfer of energy from the beating cilia to the viscoelastic mucus layer requires that the ciliary beat frequency be faster than the relaxation time (*56*). This requirement is easily met, since typical ciliary beat frequencies range from 7

cycles/sec in the peripheral airways to 25 cycles/sec in the trachea (*61*). This variation in ciliary beat frequency is reflected in the above mentioned increased rate of mucus flow that takes place in the trachea. The surface area over which mucus flows in the peripheral airways has been calculated to be approximately 70 m^2, compared to 0.6 m^2 in the trachea (*10*). This means that the proportionate volume of mucus reaching the trachea is increased over 100-fold with respect to the distal airways. The higher tracheal ciliary beat frequencies are needed to prevent mucus accumulation and clogging of the airways.

Studies of the regulation of ciliary beat frequency have suggested a dual control mechanism. A direct neurohormonal effect seems to be mediated by β-adrenergic stimulus and transduced via cAMP, with an ultimate effect on the axoneme itself (*62*). It has been proposed that cAMP and Ca^{2+} regulate ciliary beat frequency by regulating the intracellular availability of ATP or its access to the axoneme (*63*). In addition to providing the chemical energy for the movement of the cilia, ATP is one of several purine nucleotides and nucleosides demonstrated to provide luminal regulatory control of ciliary beat frequency via specific purinergic receptors (*64*). A second, possibly independent, effect is based on mechanical stimulation of the cilia by foreign particles or by mucus itself. (*56,62*). Calcium flux experiments have shown that mechanostimulation of ciliary activity may involve the opening of calcium channels and the elevation of intracellular calcium concentrations (*56*). It is clear that many stimuli of mucus secretion also stimulate mucus transport, i.e., increase ciliary beat frequency. Whether the ciliary response to these stimuli is secondary to the presence of increased amounts of mucus on the cell or is independent from it is an issue yet to be resolved (*56*).

In addition to optimized ciliary beat frequencies, efficient mucus mobilization requires proper contact between the cilia and the mucus blanket. Under normal conditions, the cilia beat while completely immersed in periciliary fluid, with only the tips of the cilia coming in contact with the mucus blanket (*43*). Each ciliary beat consists of a propulsive stroke, a rest phase, and a preparatory stroke. During the propulsive stroke the cilium reaches its maximum extension, causing the ciliary crown to penetrate the mucus layer, thereby transferring its momentum to it (*44,56*). The rest phase may merely reflect a metabolic recovery period or serve as a reserve period that enables increased beat frequency when needed (*10*). Ciliary movement during the recovery period is backwards and downwards, with a clockwise rotation in a plane parallel to the cell surface (*44,56*). The combined effect of the different phases of the ciliary beat is to propel the mucus unidirectionaly, with minimal energy spent repositioning the cilium for the next stroke. The thickness of the periciliary layer provides the critical distance that allows optimal ciliary contact with the mucus layer above. The cilium itself appears to have a regulatory effect on the depth of the periciliary fluid. If the fluid is too deep, excess fluid may be swept away by ciliary beating. When the layer is too shallow, ciliary contact with the mucus layer will be stronger, and this may stimulate ionic transport by the ciliated epithelium which in turn results in increased fluid secretion into the airway (*56*).

Several techniques for the measurement of mucus clearance rates have been devised over the years, as recently reviewed by Schlesinger (*65*). In their simplest form, these methods involve monitoring the movement of endogenous or artificial markers (inhaled, blown in, or placed on the airway) from the periphery toward central areas of the respiratory tract. Artificial markers commonly used include pollen, Teflon disks, dyes, colored beads, and technetium-99-radiolabeled iron particles. Detection methods depend on the type of marker used, but typically involve bronchoscopy, serial sampling, or imaging with a gamma camera. An elegantly simple method for measuring mucus velocity in the nose is the saccharine test, in which the time elapsed before a subject is able to taste a saccharine tablet placed in his nose is measured (*44,65*).

Additional factors affecting mucociliary transport include gravity and air flow (*10*). Gravity can impair the efficiency of ciliary activity significantly when the thickness of the periciliary layer is greater than 10 μm or when the fluid becomes less viscous due to dilution. In large airways, turbulent airflow can also contribute to mucus clearance (*66*). This is in fact the mechanical basis for the cough reflex, the only alternative mechanism for removing mucus from the airways when mucociliary clearance is impaired (*10*).

Mucin Biochemistry

Given that glycoproteins are responsible for the physiologically relevant characteristics of mucus, it has naturally followed that research efforts directed at understanding the metabolic and genetic aspects of airway mucus biology have focused on mucins. Accordingly, the following section will consist of a review of current mucin biochemistry and molecular biology.

Mucin Genes and Transcription

Present understanding of the organization and regulation of genes coding for mucin peptides, or apomucins, is rudimentary, as this field is very much in its developing stages. Human tracheobronchial apomucin genes have been mapped by Aubert and colleagues to chromosomes 11p15, 13, and 3 (*33,67*). In addition, a cystic fibrosis tracheal apomucin gene has been mapped to 11p13-11Ter (*68*). The presence of multiple nucleotide sequence homologies between chromosomes 11 and 13 have hampered efforts to determine whether there are multiple apomucin genes on chromosome 11p15. However, at present, at least two or three other chromosomes are implicated as containing apomucin genes.

Only partial cDNA sequences of tracheobronchial apomucins have been published in the literature to date. Aubert and co-workers screened a λ-gt11 cDNA library from human bronchial mucosa with antibodies prepared against chemically deglycosylated airway glycoproteins. Immunohistochemical studies carried out with these antisera showed specific labeling of goblet and mucous glands that was limited to the perinuclear area and did not include mucus granules, which contain the highly glycosylated product (*46*). Upon screening of the cDNA library with these antisera, the nucleotide sequences of positive clones were examined (*33*). This work has so far produced three families of human tracheobronchial glycoprotein genes, suggesting that human airway apomucins are considerably more heterogeneous than once was thought. The first family is described as consisting of repetitive sequences of 8 and 16 amino acids. This pattern of multiple repeated sequences appears to be a characteristic of apomucin genes. The second family contains two clones with near identical amino and carboxy termini that share perfect homology with 14 of a 22 amino acid stretch of a human tracheobronchial apomucin previously published by a separate group (*69*). Furthermore, this second family contains a third clone that has sequences coding for 30 uninterrupted hydroxy amino acids. The third family described by Aubert et al. features hydrophobic and hydrophilic regions arranged in alternating patterns. All

of the tracheobronchial mucin cDNA sequences described by Aubert's group exhibit a repeated pattern consisting of a domain of hydrophilic amino acids flanked by a histidine-rich sequence and a proline-rich sequence. Presumably, these flanking sequences code for nonglycosylated and glycosylated regions of the peptide, respectively. A partial sequence of canine tracheal mucin cDNA has also been reported (*70*).

In addition to the extensive heterogeneity of apomucin genes, there is also much variation at the transcriptional level. Northern hybridization of mucosal epithelium with mucin probes produces smears instead of discreet bands, indicating that there is extensive heterogeneity in the size of mucin mRNAs (*71–74*). On the basis of sequence alignments and comparisons, Aubert and colleagues have reported that tracheobronchial mucin exons are relatively small and may be derived from complex alternative splicing (*33,46*). For detailed information about mucin peptide genes, a recent review article is available (*34*).

Glycosylation

Although mucin carbohydrate structure is very heterogeneous, it can be understood by noting some simple rules. For example, there are three general domains to mucin glycans: the core (not to be confused with the peptide core), the backbone and the periphery (*20,74,75*). The core is simply the first two or three sugar residues that are bound to the *N*-acetylgalactosamine at reducing terminus.

The four major core structures found in airway mucins are: Core 1, Gal(β1–3)GalNAc(α-O)Ser/Thr; Core 2, Gal(β1–3)-(GlcNAc(β1–6)GalNAc(α-O)Ser/Thr; Core 3, GlcNAc(β1–3)GalNAc(α-O)Ser/Thr; Core 4, GlcNAc(β1–3) GlcNAc(β1–6)GalNAc(α-O)Ser/Thr.

The four backbone structures are: Type 1, Gal(β1–3)GlcNAc; Type 2, Gal(β1–4)Glc-NAc; i antigen, GlcNAc-(β1–3)Gal; and I antigen, GlcNAc(β1–3)-(GlcNAc(β1–6) Gal (I-Step) and GlcNAcβ6Gal/GalNAc (I-Ma) (*76*).

The carbohydrate structures of the peripherals are rather complex. For example, sialic acid can link to C-3 or C-6 of Gal and C-6 of reducing terminal GalNAc while sulfate has been identified at C-6 of Gal (*37,38*).

Substitutions at the nonreducing terminal type 1 chain can form blood group H, Fucα1–2Gal; B, Gal(α1-3)Fuc(α1–2)Gal; A, GalNAc(α1–3)Fuc(α1-2)Gal; Lewis a (Le^a), Gal(β1–3)(Fuc(α1–4)GlcNAc; Lewis b (Le^b), Fuc(α1–2)Gal(β1–3)Fuc(α1–4)-GlcNAc; and sialyl Le^a, NeuAc(α2–3)-Gal(β1–3)Fuc(α1-4)GlcNAc.

Substitutions at terminal type 2 chain can form Lewis x (Le^x), Gal(β1-4)Fuc-(α1–3)GlcNAc; Lewis y (Le^y), Fuc(α1-2)Gal(β1–4)Fuc(α1–3)GlcNAc; and sialyl Le^x, NeuAc(α2–3)Gal(β1–4)Fuc(α1–3)-GlcNAc in addition to blood groups H, B, and A determinants. Fuc also links α1–2 to internal Gal, a structure recently identified (*38*).

Thus, mucin glycans alone can vary extensively in their overall structure, based on the possible permutations of the cores with the different backgrounds and possible peripheries. When one considers that the mucus glycoproteins consist of peptide chains covered in sections with this vastly heterogeneous spectrum of mucin glycans, it can be readily appreciated that these are very large and highly complex molecules. For further information about airway mucin carbohydrate structures, several comprehensive reviews have been published (*20,22,37,75*).

As with any peptide, apomucin translation takes place in the rough endoplasmic reticulum and involves the initiation, elongation and termination of the peptide product. The addition of the first sugar, *N*-acetylgalactosamine, occurs at the cis-Golgi compartment (*77,78*). Other glycosylation steps as well as sulfation reactions take place at various compartments of the Golgi apparatus as demonstrated by cytochemical, histochemical and autoradiographic approaches (*79*). Assembled mucin glycoproteins are then packaged inside vesicles formed from the Golgi lamellae. Mucin secretory granules are made up of coalesced vesicles, and migrate towards the apical aspect of the cell where they release their contents into the extracellular environment (*80*).

Mucin oligosaccharide synthesis proceeds one at a time as catalyzed by glycosyltransferases, which transfer sugars from nucleotide sugars to the acceptors. Nucleotide sugars are generated in the cytoplasm and then transported into the Golgi lumen by specific transporters (*81*). Assembly of mucin carbohydrate is subjected to the control of several factors (*82*), including availability of nucleotide sugars that serve as donors, relative activities of the glycosyltransferases acting on the same acceptor, the mucin peptide sequence, presence of activator or inhibitor, nucleotide sugar transporter (*81*), and pH in the lumen of Golgi apparatus (*83*). The product formed in the preceding reaction will then serve as the acceptor for subsequent glycosyltransferase reactions. In other words, the assembly of mucin carbohydrates is not directed by a template, but by various glycosyltransferase-catalyzed reactions. The glycosyltransferases involved in the assembly of mucin oligosaccharides can be classified into the chain-elongation and chain-termination enzymes. The chain-elongation enzymes generate the carbohydrate structures which allow for continued growth of the carbohydrate chains. This group of enzymes includes mucin peptide:αGalNAc transferase (*77,84*), βGal transferases (*85–87*), and βGlc*N*Ac transferases (*88–90*). On the other hand, the carbohydrate structures generated by the chain-termination enzymes stop or limit further chain growth. These enzymes include sialyltransferases (*91*), and blood groups A (*92*), B (*93*), and H (*94*) enzymes. If sialic acid is linked to the first sugar, i.e., GalNAc, the product can not accept additional sugars. Depending upon the sequence of addition of these sugars as catalyzed by these two groups of enzymes, mucin carbohydrate chain length and structures can be very heterogeneous. This phenomenon could explain the tremendous microheterogeneity exhibited by mucin oligosaccharides. When mucin synthesis nears completion, sulfation of the oligosaccharides takes place at the trans-Golgi membranes (*81*) before mucin is packaged into the secretory granules. Sulfotransferase-catalyzed reactions utilize the sulfate donor, 3'-phospho-5'-phosphoadenosylsulfate (PAPS). PAPS is formed in cytoplasm from inorganic sulfate, which is derived from serum inorganic sulfate, or from sulfur-containing amino acids, by the consumption of two molecules of ATP as sequentially catalyzed by ATP sulfurylase and adenosine 5'-phosphosulfate kinase (*95,96*).

Glycoprotein Release

As reviewed by Verdugo, there have been several recent advances in our understanding of the complex processes that lead up to the release of mucins from the cell, and the formation of the continuous mucus gel that covers the airway epithelium (*97*). The attachment of a secretory granule containing mucins in a condensed state to the plasma membrane at the apical end of the epithelial cell is the first step in mucin exocytosis. The expansion of a fusion pore on the plasma membrane leads to increased water and ionic permeability and release of the granule contents into the airway. Until relatively recently it was thought that

membrane tension established by osmotic swelling of the secretory granule was responsible for the formation and widening of the pore. However, new evidence has shown that pore formation precedes, and can be uncoupled from, granule swelling (*97*).

The explosive rate of swelling undergone by mucins upon exocytosis cannot be accounted for by a simple osmotic process. Moreover, condensed mucin granules fail to decondense when placed in water free of ions (*98*). These and other findings have led to the "jack-in-the-box" model of glycoprotein exocytosis (*97*). This theory proposes that glycoprotein exocytosis is the result of rapid swelling of the glycoprotein polymer inside the secretory granule. While inside the granule, glycoproteins are in a condensed state that is maintained by the presence of a shielding species, such as calcium ion in the case of mucin. Increased permeability due to pore formation and widening results in the exchange of calcium for sodium inside the granule. This causes the mucin molecules to undergo a rapid phase transition to a hydrated, decondensed state (i.e., to swell) and be released.

Once released, airway mucins serve as the key rheologic assembly component of airway mucus. As pointed out by Verdugo, it is the ability of mucins to form entanglements rather than crosslinked networks which allows mucus to expand in an unconstrained manner while absorbing water to form the mucus gel. Determinants of mucus hydration include water availability, the concentration of ions and polycations, and the pH of the airway liquid (*97*).

Physiologic and Pharmacologic Control of Mucus Release

Neurogenic Control

Basal production and release of airway mucus in humans is believed to be spontaneous and independent of enervation, since neurotransmitter antagonists and vagotomy have no effect on secretion (*99*). Furthermore, tracheobronchial explants continue to secrete mucus *in vitro* (*3*). However, stimulation of mucin production above basal levels by submucosal glands is clearly under autonomic control. Parasympathetic control is evidenced by studies showing that electrical stimulation of the vagus nerve results in increased mucus secretion by submucosal glands. Vagal control of submucosal glands is cholinergic, since it can be reproduced with cholinergic agonists like pilocarpine and blocked with cholinergic antagonists such as atropine (*100*).

There is also evidence of sympathetic control of airway mucus secretion via beta receptors (*54*). α and β adrenergic sympathomimetic agents have specific effects on submucosal glands. Mucus and serous cells are stimulated through α receptors to secrete mainly fluid and some glycoprotein. In contrast, specific β agonists target mucus cells to release chiefly glycoprotein. This implies that mucus composition is at least partly under autonomic control, and is thus susceptible to pharmacologic intervention (*3*).

As reviewed by Phipps (*3*), airway mucus secretion can also be stimulated via reflex mechanisms. Both fluid and glycoprotein secretion by submucosal glands can be increased by mechanical and chemical irritation of the airway epithelium. Reflex-mediated release of glycoprotein from submucosal glands involves, but may not be limited to, sympathetic and parasympathetic enervation.

Humoral Control

A variety of bioactive peptides and lipids also stimulate mucus secretion in the airways under certain experimental conditions. Histamine is effective in the cat and goose trachea (*100*), but not human airways in vitro (*55*), while substance P and kallidin are stimuli of the dog and rat trachea (*80*). Prostaglandins A_1, E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$ are also stimuli of tracheal mucin secretion (*80,101*). The potent phospholipid inflammatory mediator platelet activating factor also stimulates mucin secretion of tracheal explants *in vitro* (*102*).

Mechanism of Glycoprotein Release

Relatively little is known about the cellular and biochemical mechanisms that mediate the release of glycoprotein in airway cells. As reviewed by Spicer and Martinez, there is evidence to suggest that the mechanisms are similar to those of other exocrine glands (*80*). Cyclic nucleotides stimulate mucin release in rat tracheal explants, suggesting their role in a signal transduction mechanism. Similarly, the phosphodiesterase inhibitor, theophiline, causes glycoprotein secretion in human airways in vitro (*100*). Calcium dependency, possibly with a role in signal transduction, has also been demonstrated in rat trachea stimulated with acetylcholine (*80*).

Differential Control of Glycoprotein Release

The study of selective release of glycoprotein from submucosal glands and goblet cells in the airways is complicated since both are present in airway tissue. Recently, studies of goblet cells in tissue culture have yielded insightful information, as reviewed by Kim (*103*). While stimulation of glycoprotein release from submucosal glands is neurogenic, goblet cells appear to be under local humoral control (*100*). No efferent enervation occurs above the level of the submucosal glands of the airway (*42*). Furthermore, direct neurogenic stimulation, such as stimulation of the vagus nerve, does not alter mucin release in the goblet cell (77). Consistent with these findings, many effective submucosal autonomic agents that stimulate mucin release in explants have no effect on goblet cells in vitro. These include acetylcholine, norepinephrine, and isoproterenol (*100*). This contrast in the pharmacology of mucin release by submucosal glands and goblet cells seems to extend to proinflammatory humoral mediators as well. For example, prostaglandins E_2 and $F_{2\alpha}$ have no effect on goblet cells *in vitro*, yet they induce mucus secretion in animal trachea (*80,103*).

Release of glycoproteins by goblet cells in vitro can be stimulated by ATP, proteases, or by physicochemical manipulations, such as alteration in pH, mechanical stress, and hypoosmolarity (*103*). The response to ATP is receptor mediated and specific. In contrast, glycoprotein release induced by physicochemical stimuli, such as pH changes, is believed to be the result of damage of the cell membrane. Hypoosmotic conditions are analogous to mechanical stress on the cell in that both types of stimulus seem to work through pressures exerted on the cells. Similarly, the effect of mast cell and neutrophil proteases (e.g., elastase, chymase, and cathepsin G) may also be nonspecific, being the result of cleavage of membrane-bound glycoproteins as well as damage caused by hydrolysis of cell membrane proteins (*103,104*). For additional information regarding mechanisms that control mucus release, the reader is refered to an excellent review by Lundgren and Shelhamer (*105*).

Mucus in Lung Disease

Pathophysiologic changes involving airway mucus are a feature of many pulmonary diseases. These changes can be either qualitative, i.e., changes in mucus composition or structure, or quantitative, i.e., changes in the amount of mucus in the lung. A

change in mucus composition could be due to altered glycoprotein biosynthesis, electrolyte transport, or water content. Alternatively, structural modifications can be the result of interactions between normal mucus and pathogens or reactive chemicals.

Typically, quantitative changes in mucus involve hypersecretion of airway mucin. The pathogenesis of mucus hypersecretion in the airway was recently reviewed (*105*). Hypersecretion can be due to increased mucin biosynthesis and release by goblet cells or submucosal glands, or to increased numbers of mucin-secreting cells brought about by faster cell division (hyperplasia) or by differentiation of nonsecreting cells (metaplasia) (*106,107*). Another mechanism leading to increased mucus volume in the lung is leakage of plasma components into the airspace as a result of increased vascular permeability (*107*). A brief review of pulmonary diseases in which some of these underlying mechanism contribute to the pathologic role played by mucus now follows.

Chronic Bronchitis

Chronic bronchitis is basically an inflammatory disease of the airways characterized by persistent chronic cough with sputum production. Most patients with chronic bronchitis are smokers or ex-smokers (*108*). Microbial and viral infections, as well as urban and industrial air pollutants, are believed to exacerbate the condition, but are not likely initiators of bronchitis (*106*). The pathologic hallmark of chronic bronchitis consists of hypertrophy and hyperplasia of submucosal glands, as well as hyperplasia and metaplasia of goblet cells (*105,106*). Mucus hypersecreted in chronic bronchitis is apparently normal in composition, although it can contain a higher content of plasma components which may inhibit ciliary activity (*108,109*). Mucus from bronchitic patients is more viscous during flare-ups of the disease. In addition, persistent reductions in the rate of mucociliary clearance have been reported in patients with chronic bronchitis (*108*).

Cystic Fibrosis

Cystic fibrosis is a disease that illustrates in compelling terms the importance of mucociliary clearance to normal lung function. The most common lethal genetic disease among Caucasians (*9*), cystic fibrosis is characterized by a systemic defect in exocrine gland secretion. Expression of the disease varies considerably, but sweat and mucus glands are involved most frequently. The most serious manifestation of the disease is the retention within the airways of abnormally viscous mucus (*106*). It is the inability of the mucociliary system to remove this thick mucus, along with trapped microorganisms and debris, that results in enlargement of airway caliber and persistent infection. Persistent infections with *Staphylococcus aureus* and *Pseudomonas aeruginosa* are characteristic of cystic fibrosis and lead to chronic inflammatory reactions which are believed to be responsible for much of the destruction of lung tissue that is seen in patients afflicted with the disease (*106,110*). The inflammatory response is multistaged and complex, involving the participation of neutrophils, monocytes and macrophages. Proteolytic destruction of lung tissue by neutrophil-derived proteases such as elastase is thought to be a principal source of lung damage in cystic fibrosis (*110*). Increased sulfation of airway mucins (*24*) and other high molecular weight glycoconjugates (*111*) has been reported. However, the relationship between altered sulfation of glycoconjugates to the basic defect, mucus accumulation, and bacterial colonization in cystic fibrosis patients remains to be elucidated.

As reviewed by Welsh, the central pathogenic feature of cystic fibrosis is an alteration in electrolyte transport by the airway epithelium, which leads to insufficient hydration of glycoproteins (*9*). Airway epithelial cells secrete Cl^- into the airway and absorb Na^+ from the airway. Cl^- and Na^+ ions are first co-transported into the basolateral side of the cell in an electrically neutral process that allows intracellular transport of Cl^- against its concentration gradient. This process is made possible by the action of a Na^+/K^+ATPase, which exports Na^+ through the basolateral membrane of the cell, thus keeping its intracellular concentration low. This ATPase activity also moves K^+ into the cell, which indirectly contributes to a favorable gradient for apical Cl^- secretion and Na^+ absorption through specific regulated channels. Many neuropeptides (e.g., bradykinin), hormones (e.g., aldosterone) and mediators (prostaglandins) influence the permeability of the Cl^- channel(s), apparently by increasing cAMP levels in the cell (*9*).

Airway epithelial cells from cystic fibrosis patients secrete considerably less Cl^- ion and absorb more Na^+ through their apical membrane than cells from normal subjects (*9,121*). This is due, at least in part, to a defective cAMP-regulated Cl^- transport channel (*9,113*). The putative gene responsible for this defect was identified in 1989 (*114–116*). The gene coding for "cystic fibrosis transmembrane conductance regulator" (CFTR) has a deletion of 3 base pairs corresponding to phenylalanine at residue 508. This single mutation in CFTR accounts for 70% of cystic fibrosis genes (*116*). The altered CFTR peptide causes abnormal glycosylation of CFTR, rendering its failure to be targeted to the plasma membranes at 37°C (*117*). This targeting abnormality can be reversed at 27°C (*118*). CFTR has been shown to be a chloride channel (*119*); however, other physiological functions of CFTR remain to be identified.

Asthma

Bronchial asthma is essentially a chronic respiratory disease that manifests itself intermittently as attacks of dyspnea (shortness of breath) and wheezing caused by bronchial spasms. Nearly all characteristics of the disease such as its severity, course, aggravating factors and frequency and duration of attacks, vary widely among patient. Most patients have a familial predisposition to atopic disease. Several types of asthma are recognized based on apparent etiologic factors. These include exercise asthma, cold air asthma, and industrial (chemically induced) asthma (*106*). While the basic mechanism of the disease is still poorly understood, all asthma attacks seem to involve a predisposing airway hyperreactivity and the release of a battery of inflammatory mediators that cause bronchoconstriction and mucus hypersecretion (*105,106*). A great deal of attention has been focused on the possible link between air pollution and an alarming rise in the number of cases of fatal asthma attacks (*120*).

Gross pathology findings in the airways of victims of fatal asthma attacks (status asthmaticus) include the presence of a tenacious mixture of mucus, exudate, epithelial cells, lymphocytes, and eosinophils (*48*). Mucoid impactions in the airways of asthmatics may be present even in the absence of infection (*121*). Also evident are hyperplasia and hypertrophy of submucosal glands and hypertrophy, hyperplasia, and metaplasia of goblet cells in peripheral airways (*106*). The same histologic changes are reported in patients with stable asthma, but to a lesser degree (*122*). Mucus plugs and impaired mucociliary clearance may be found even in patients with mild asthma or during remission of the disease (*123,124*). In addition to hypersecretion, changes in mucus secretion in bronchial asthma include increased permeability to serum

constituents and altered water transport into the airway (*122*).

Increased endothelial permeability is believed to mediate the airway edema observed in cases of fatal asthma. Similarly, increased epithelial permeability is likely to be responsible for the leakage of serum components into the airway, changes in the periciliary fluid layer that can lead to inhibition of ciliary function, and increased mucus volume in the lung (*109,122*). In addition, changes in water transport by the airway epithelium, possibly mediated by histamine, have been reported in experimental asthma models and may also contribute to these effects (*122*).

Sputum Analysis

Mucus samples are often obtained for clinical analysis and diagnosis as expectorated sputum. Sputum is a chemically unstable mixture of mucus, saliva, surfactant, cells, and plasma constituents that is not present in the normal lung (*109*). A further complication of inferences made from sputum analysis is the need to distinguish between purulent and nonpurulent sputum. Purulent sputum is recovered from patients with an overt or underlying infection and usually has increased amounts of glycoprotein, DNA, and a higher content of plasma constituents than nonpurulent sputum (*109*). In spite of these limitations, sputum samples can yield useful information about pathologic changes involving mucus production in disease. For instance, an increase in the *N*-acetylneuraminic acid:fucose ratio in the sputum is understood to reflect leakage of plasma constituents into the airway, which in turn, may indicate a relatively more severe inflammatory reaction in the airways. An elevated *N*-acetylneuraminic acid:fucose ratio is observed in sputum samples obtained from patients with asthma; however, the absolute concentration of these compounds is low. This suggests that plasma constituents contribute significantly to the increased volume of mucus present in asthmatic lungs. Sputum obtained from bronchitis patients, on the other hand, has high amounts of *N*-acetylneuraminic acid and fucose but the ratio is normal, indicating that the increased amount of mucus produced in bronchitis is mainly the result of hypersecretion. In sputum from patients with cystic fibrosis the reverse is found, i.e., relatively normal amounts of *N*-acetylneuraminic acid and fucose but a high *N*-acetylneuraminic acid:fucose ratio. This suggests that in cystic fibrosis, there is an inflammatory process that results in leakage of plasma components into the airway without hypersecretion of mucus (*109*).

The DNA content of sputum is a similar marker of the severity of lung inflammation. Normal lung secretions and nonpurulent bronchitis sputum do not contain detectable amounts of DNA. In contrast, purulent bronchitis sputum contains detectable amounts of DNA, and even nonpurulent cystic fibrosis sputum contains significant quantities of DNA. This finding may well attest to the relative severity of airway inflammation that occurs in cystic fibrosis (*109*). Human recombinant DNase has been examined as a possible therapeutic means to alleviate the symptoms of CF patients (*125*).

Morphologic Changes in Disease

Although hypertrophy of submucosal glands is evident in both bronchitis and asthma, a relatively greater increase in mucus acini compared to serous acini is seen to occur in bronchitis, but not asthma, patients (*48,126*). Since the serous acini of submucosal glands secrete antibacterial and antiprotease compounds in addition to glycoprotein, the dilution of these components by an increased volume of mucus could result in diminished resistance to both infection and proteolytic attack in bronchitis (*48*).

The mechanism responsible for the development of submucosal gland hypertrophy and hyperplasia in asthma and bronchitis is presently unknown. However, neutrophil-derived proteases such as elastase have been implicated in goblet cell hyperplasia, as reviewed (*105,107*). These authors have also proposed a possible mechanism wherein chemotactic lipid mediators such as leukotriene B_4 are secreted by epithelial cells during the initial stages of airway inflammation. These mediators could recruit neutrophils into the airway which could then release elastase, causing goblet cell hyperplasia. In support of this scenario is the finding that glucocorticoids, which inhibit the synthesis of lipid inflammatory mediators, can prevent goblet cell hyperplasia induced by neutrophil products (*127*).

Effects of Air Pollutants on Airway Mucus

Inhalation of a variety of ambient and occupational air pollutants is known to result in a number of untoward effects in the lung. These include changes in pulmonary function, diminished lung defense and impaired mucociliary clearance, as well as obstructive, inflammatory, and neoplastic disease. These topics have been the subjects of several excellent recent reviews *(128–132)*. This section will focus on specific effects of air pollutants on airway mucus function, secretion, and biosynthesis, as they pertain to the development of pulmonary toxicology and disease.

Effects on Mucociliary Function

Impairment of mucociliary function is a consequence of exposure to a variety of air pollutants. The effects of inhaled toxicants on respiratory tract clearance mechanisms have been reviewed by Schlesinger (*65*). Essentially, mucociliary targets of inhaled air pollutants are the ciliated epithelium, the periciliary fluid, and the mucus layer.

In addition to the outright epithelial desquamation or destruction of cilia induced by high concentrations of SO_2, NO_2 and O_3 (*133,134*), ciliary activity in the airway epithelium is susceptible to alteration by toxic agents. Agents that impair ciliary beat rate (i.e., induce ciliary dyskinesia or ciliostasis) include H_2SO_4 (*135*), SO_2, NO_2, ammonia (*136*), wood dust, cadmium (*137*), nickel, hairspray (*138*), cigarette smoke (*139*), and formaldehyde (*140*). Interestingly, Grose and colleagues found that prior exposure to O_3 diminishes the ciliostatic effect of H_2SO_4 in an *ex vivo* system (*135*). The mechanism of action of ciliostatic compounds can involve structural damage of the cilium, as induced by NO_2 (*141*), or altered energy metabolism caused by heavy metals (*65,138*).

In spite of these findings, the relevance of the ciliostatic effect of inhaled pollutants as a mechanism responsible for impaired mucociliary activity is thrown into question by two factors. First, it has been noted that the dose of toxicant required to induce ciliostatic changes usually far exceeds that required to produce a reduction in mucociliary clearance (*65,142*). Second, a study conducted by Battista and colleagues, showed that 10% of the ciliated epithelium in chicken trachea could carry out 30 to 50% of particle transport activity seen in control animals, suggesting that ciliary activity is present in large excess of that needed for normal ciliary function (*143*). Thus changes involving mucus production and function may be more likely mechanisms of the impaired mucociliary clearance induced by inhaled toxicants (*142*).

Physicochemical Alterations

Another way in which an inhaled pollutant can alter mucociliary clearance is by altering mucus rheology, either by interacting with mucus constituents directly or by

influencing its biosynthesis. An alteration of mucus rheology, such as a decrease or increase in viscosity, can diminish the efficiency with which energy is transferred from the beating cilia to the mucus blanket. As presented by Holma (*144*) variations in mucus pH, such as those caused by SO_2 can have a profound effect on mucus rheology. Although factors such as protein concentration and ionic strength also come into play, a reduction in pH generally increases mucus viscosity (*144*). Glycoproteins appear to be the principal acid-reactive component in mucus, and, in fact, have been demonstrated to be largely responsible for the buffering capacity of mucus (*144*).

Schlesinger (*65*) discusses chemical cross-linking of glycoproteins as a mechanism via which inhaled toxicants can alter mucus viscosity. Exposure to formaldehyde, a compound known to form chemical cross-links in proteins and nucleic acids (*145*), reportedly produces increased mucus viscosity (*146*). Similarly, reduced viscosity resulting from exposure to O_3 is proposed to be caused by a decrease in the number of chemical cross-links (*65,147*). However, Verdugo has recently argued that the rheologic properties of mucus are imparted not by cross-links between glycoprotein moieties, but by networks of entanglements between glycoprotein strands (*97*). Thus, while formaldehyde may indeed increase mucus viscosity by forming cross-links, the reduction in mucus viscosity may not be due to the destruction of existing cross-links between glycoproteins.

Biosynthetic Alterations

Certain inhaled toxicants induce qualitative changes in glycoprotein biosynthesis. Exposure to H_2SO_4, SO_2, or cigarette smoke induces a shift in the type of glycoprotein secreted by the airway to produce a relatively more acidic mucin (*18,19,64*). This phenomenon is also seen in chronic bronchitis and cystic fibrosis, where the degree of sulfation of secreted glycoproteins is increased (*24,91,148*), suggesting that it is an adaptive response to cellular injury. In addition, elevation of the activities of several glycosyltransferases has been observed in airway epithelium of SO_2-exposed dogs and patients with chronic bronchitis (*149,150*). With cigarette smoke, the increase in acidic mucin seen in submucosal glands and goblet cells is due to an increase in sialic acid-containing mucins (*18*). Normal rat tracheal epithelium preferentially secretes neutral glycoprotein, while in the peripheral airways there is a bias for the production of acidic glycoprotein. Acute exposure to cigarette smoke abolishes these regional differences within 24 hr, resulting in dominance by a cell that produces both acid and neutral glycoprotein throughout the rat respiratory tract. Sustained exposure to cigarette smoke eventually results in a population of cells that produces only acidic glycoprotein (*18,148*). Unlike goblet cell hyperplasia, the change towards secretion of acidic mucin is apparently not blocked by the anti-inflammatory compound phenylmethyloxadiazole (*18*). Whether increased secretion of acidic mucin is due to secretion of molecules with a higher acid content or more molecules with acid moieties is unclear (*148*). Interestingly, induction of acid glycoprotein secretion was not observed using O_3 as a toxicant in recent studies by Hotchkiss and associates, who found an increase in both acid and neutral glycoprotein in rat and primate nasal epithelium (*151,152*). Whether this reflects a difference in the response by nasal and tracheobronchial epithelium or in the nature of the stimulus is not clear.

Effects on Ciliary Fluid

The depth of the periciliary fluid layer largely determines the quality of the interaction between the cilia and the mucus blanket over the airways above. Optimal periciliary fluid depth and composition are necessary for efficient transfer of energy from the tips of the cilia to the mucus during the propulsive stroke, and for the recovery stroke to take place unimpaired (*56*). Periciliary fluid depth and composition can be affected by compounds that affect ion transport by the airway epithelium or increase epithelial permeability to serum components, which can affect mucus rheology and secretion of glycoproteins (*19,65,153*). For example, in vitro exposure to O_3 results in increased Na^+ ion permeability across guinea pig airway epithelium (*154*), while O_3 inhalation produces increased water and Cl^- ion secretion in sheep tracheal explants *in vitro* (*153*). Similarly, O_3 inhalation causes increased epithelial permeability to macromolecules in humans (*155*). Foster et al. found that increased secretion of airway fluid or an alteration of epithelial permeability could be responsible for the alterations in mucociliary function that they reported in humans exposed to O_3 (*156*). Exposure to NO_2 caused increased mucosal permeability to large protein molecules in guinea pig lungs (*19*). Increased epithelial permeability to water and serum components is also a feature of the pathology of cystic fibrosis and asthma (see preceding section).

Mechanisms of Hypersecretion

Mucus hypersecretion is an important part of the sequelae induced by a number of toxic insults to the lung. Possible mechanisms of hypersecretion include increased release of existing glycoprotein stores, hypertrophy of submucosal glands, hyperplasia of goblet cells, metaplasia of nonsecreting cells into goblet cells, and an altered rate of mucin biosynthesis by goblet cells or cells in the submucosal glands.

Inhalation of some toxic compounds produces acute mucus secretion into the airways. For example, NH_3, (*54*), and O_3 (*19,153*) induce glycoprotein discharge from glycoprotein-secreting cells in the airways. Exposure to acidic and alkaline media also stimulate mucin release, albeit secondary to cell membrane damage (*157*). Similarly, inhalation of dusts (e.g., charcoal or barium sulfate) provokes release of mucus from cat tracheal explants via a neurogenic reflex pathway as well as a direct stimulation of the mucosa (*19*). An increase in tracheal gland sensitivity to cholinergic stimulation has been reported in ferrets exposed to O_3 (*158*). Both myelinated and unmyelinated (C-fibers) neuronal pathways may be involved in the discharge of mucus in the airway (*3,64,105*). Eicosanoids such as leukotrienes C_4 and D_4 and hydroxyeicosatetraenoic acids, have also been shown to mediate glycoprotein release from human airway explants (*159,160*), and may be involved in mucus secretion in response to toxicant exposure (*153*). A study by Jones et al. showed that acute exposure of rats to cigarette smoke causes extensive degranulation of mucus-secreting cells, to the point that there is a transient decrease in the number of cells staining positive for glycoprotein content in the airways (*148*). In the same study, the anti-inflammatory phenylmethyloxadiazole did not prevent tobacco smoke-induced discharge of mucus-secreting cells. However, in a similar study the same drug was shown to be effective in preventing an increase in the basal rate of mucin discharge induced by tobacco smoke, possibly reflecting a difference in the time of administration of the drug between these studies (*161*). The balance between glycoprotein synthesis and release in secretory cells is reportedly altered as a result of chronic inhalation of tobacco smoke or SO_2. This is evidenced by a decrease in the number of intracellular glycoprotein granules in exposed cells, sug-

gesting a higher rate of secretion of glycoprotein granules relative to their storage time inside the cell (*80,148*). Work by Mariassy and colleagues has shown that O_3 exposure can affect developmental changes in the respiratory epithelium of postnatally exposed lambs. Their studies demonstrate that the decreased secretion of total and sulfated glycoproteins that normally accompanies maturation is retarded in O_3-exposed lambs, possibly leading to impaired mucociliary function in these animals (*162*).

Increases in the number of airway mucin-secreting cells can be induced by inhalation of SO_2 (*163*), O_3 (*153*), Cl_2 (*164*), NO_2 (*165*), or cigarette smoke (*166*). SO_2 and tobacco smoke-induced hyperplasia have been studied as animal models of bronchitis. As reviewed by Spicer and Martinez (*80*) and Abraham (*19*), submucosal gland cell and goblet cell hyperplasia is readily apparent in the airways of dogs and rats chronically exposed to SO_2. The response of the goblet cell population in the bronchi and bronchioles of dogs exposed to SO_2 is characterized by an increase in both size and number (*163*). The expansion of the goblet cells population is partially due to metaplasia of other epithelial cell types into gobletlike cells with enhanced mucus-secreting capabilities (*80*). Submucosal gland hypertrophy is apparently also due to both increased replication rate and a metaplastic change of serous cells into mucus cells (*73,80*). SO_2 inhalation in dogs has also been shown to cause elevated glycosyltransferase activities in the homogenates of tracheobronchial epithelia, although it was not possible to determine whether this was merely a reflection of the histologic changes also observed in these animals (*150*). A recent study suggested that there is an induction in mucin mRNA levels in the airways of rats chronically exposed to SO_2 (*73*). These results suggest that chronic exposure of animals to SO_2 enhances the expression of both mucin and glycosyltransferase genes, resulting in hyperproduction of mucins.

Exposure to cigarette smoke also produces submucosal gland hypertrophy, goblet cell hyperplasia, and evidence of serous-to-mucus cell metaplasia (*73,161,166*). Tobacco smoke-induced hyperplasia of secretory cells can occur within hours of a single exposure and may also involve basal cells (*148*). Metaplastic changes in the airways of rats exposed to cigarette smoke are evidenced by increased rates of mitosis among basal cells and serous cells but not mucus cells (*73*). Tobacco smoke-induced secretory cell hyperplasia in rats can be inhibited with indomethacin and steroidal anti-inflammatory drugs (e.g., dexamethasone, hydrocortisone), suggesting that cyclooxygenase products are involved (*166*). Mucolytic drugs such as *N*-acetylcysteine are also inhibitors of the hyperplastic response, perhaps by preventing glutathione depletion in cigarette smoke-exposed cells (*166,167*).

O_3 exposure also induces hypertrophy of submucosal glands and hyperplasia of goblet cells (*153*). In this study, the morphologic changes induced in the airways of sheep by chronic O_3 inhalation were correlated with decreased glycoprotein and increased water secretion. However, increased glycoprotein secretion with continued water secretion was observed following a period of recovery after exposure, suggesting that the initial decrease in glycoprotein secretion was due to depletion of mucin stores (*153*). Similar kinetics, i.e., decreased glycoprotein secretion followed by a rebound to increased secretion, was seen in the tracheal explants of rats exposed to O_3 *in vivo* (*168*).

It has been proposed that metaplasia may play a larger role in toxicant-induced hypersecretion than previously thought (*73,151*). Evidence for this hypothesis is based on studies showing increased numbers of goblet cell in areas of the lung where they are normally scarce or absent, such as the lung periphery (*169*), and an increase in the population of mucus cells without an apparent change in the mitotic rate of these cells (*170*). Hotchkiss and associates have demonstrated similar evidence of metaplastic changes responsible for the increased number of goblet cells in nasal epithelium from rats exposed to O_3 (*151*).

The possible role of inflammatory cell-derived proteases such as elastase in the development of metaplastic changes in the airway induced by tobacco smoke or SO_2 was recently reviewed by Jany and Basbaum (*73*) and Lundgren et al. (*105,107*). A study by Christensen et al. (*171*) showed that a single dose of pancreatic elastase causes an apparently irreversible goblet cell metaplasia in guinea pigs. In a more recent study, the steroidal anti-inflammatory drug dexamethasone was effective in inhibiting neutrophil elastase-induced goblet cell hyperplasia in rat trachea, suggesting the involvement of lipid mediators (*127*). Supporting this scenario is the finding that prostaglandin E_1 induces increased numbers of mucus cells, without preceding DNA synthesis, in the airways of mice, a process apparently mediated through cAMP since the analog dibutyryl cAMP had the same effect (*170*). These findings are intriguing in light of studies by Koren and colleagues, which showed that O_3 inhalation produces neutrophil infiltration into the airways, as well as increased levels of eicosanoids and neutrophil-derived elastase in bronchoalveolar lavage fluid from human subjects (*172*). Interestingly, no increase in elastase activity was found in this study. The authors suggest that this is possibly due to inactivation of elastase activity by endogenous antiproteases such as α-1-antitrypsin (*173*), and also that microfocal secretion by neutrophils could permit some elastase to escape antiproteases (*172,174*). Tobacco smoke exposure may also inhibit endogenous antiproteases in the airway (*175*).

Summary and Conclusions

Airway mucus is a glycoprotein-rich mixture secreted from the airways. It serves as a dynamic, replenishable barrier against inhaled particulate and gaseous contaminants in the lung. Vital to the role of mucus as a component of both lung defense and pathology are its viscosity and elasticity which, under normal conditions, permit mucus to act as an efficient "biologic conveyor belt," removing trapped and dissolved materials that enter the lung with inspired air. These rheologic properties of airway mucus are imparted by mucous glycoproteins synthesized and released by specialized cells in the airways.

Mucins are complex, heavily glycosylated macromolecules whose biosynthesis and secretion appear to be under close regulatory control, yet able to respond quantitatively and qualitatively to alterations in lung homeostasis, such as those resulting from respiratory disease or inhalation of toxic materials. An example of such a biosynthetic response is the increased secretion of acidic glycoproteins seen in patients with chronic bronchitis or cystic fibrosis, and in animals exposed to cigarette smoke, SO_2 or H_2SO_4. Since changes in the pH of mucus are associated with alterations in its rheology, it is tempting to speculate that increased mucus acidity is behind the increase in mucus viscosity and reduced mucociliary clearance that are also associated with these diseases and exposures.

The finding of changes in mucus pH are also intriguing in light of the fact that Holma (*144*) has suggested a link between inflammatory airway diseases such as asthma and alterations in the pH of mucus in the airway resulting from inhalation of acid aerosols. Individuals producing mucus

with low pH or low buffering capacity, e.g., some asthmatics and smokers, may have an elevated risk for developing untoward respiratory sequelae with exposure to acid aerosols. In addition to alterations in mucus rheology, acidification of mucus may lead to increased epithelial permeability, and could therefore be a mechanism leading to the airway edema seen with asthma (*144*).

Increased epithelial and endothelial permeability to serum constituents is a hallmark of inflammation in any tissue, and a pathologic feature of cystic fibrosis and asthma (*106*). Leakage of serum proteins and fluid is also thought to produce changes in mucus rheology and affect the periciliary fluid layer. One possible effect of these alterations is impaired mucociliary transport (*65*). Another consequence of the presence of serum components in the airway is the propagation of inflammatory reactions that could potentially involve dozens of known inflammatory mediators and cytokines.

The eicosanoids are one class of inflammatory mediators strongly suspected of being involved in the pulmonary response to toxic insult, possibly including those affecting mucus physiology. These oxidized derivatives of arachidonic acid are produced in response to a wide variety of cellular perturbations by a myriad of cell types, apparently each able to produce a characteristic spectrum of these compounds. Eicosanoids such as leukotrienes C_4 and D_4 and hydroxyeicosatetraenoic acids, as well as the related lipid inflammatory mediator platelet activating factor, are known stimuli of glycoprotein release (*102,159,160*) whose levels have been shown to increase in cells exposed to O_3 (*176,177*). In addition, bronchoalveolar lavage fluid from subjects exposed to O_3 contains elevated levels of various eicosanoids (*172*). Thus, it is possible that these bioactive lipids act as mucus secretagogues in response to toxicant inhalation.

An equally important role of eicosanoids may be to signal the recruitment of immune cells into the airways during the early stages of inflammation. Inhalation of some pollutants, e.g., O_3, causes the recruitment of neutrophils into the airways. Similarly, increased numbers of lymphocytes and eosinophils are found in airway secretions of asthmatics (*106*). Several eicosanoids, most notably leukotriene B_4, are potent chemotaxins for neutrophils (*107,127*), while platelet activating factor administration produces neutrophil and eosinophil infiltration into the airways (*102*). These effects are relevant to the role of mucus in the toxicology of inhaled toxicants vis-à-vis reports demonstrating morphologic changes induced by mast cell and neutrophil proteases.

The development of hyperplasia and metaplasia of glycoprotein-secreting airway tissues is a phenomenon that certain respiratory diseases and inhaled toxicants have in common. The hypertrophy of submucosal glands and hyperplasia of goblet cells seen in bronchitis, cystic fibrosis, and asthma is also induced by inhalation of O_3, SO_2,and tobacco smoke. Increased secretory capacity is the major mechanism responsible for mucus hypersecretion in the lung, and it is likely a cause of increased mucus volumes produced in response to chronic toxicant inhalation.

The mechanism by which hyperplastic and metaplastic changes are induced in the lung are unknown. However, there is growing evidence that implicates the cationic protease elastase in these responses (*107,127*). Direct instillation of elastase is known to produce goblet cell hyperplasia in the lungs of rodents. In addition, airway secretions from cystic fibrosis patients and subjects exposed to O_3 contain elevated levels of elastase which, if active, could participate in the generation of morphologic changes leading to hyperplasia and mucus hypersecretion in the airway (*104,172*). The fact that hyperplasia induced by neutrophil-derived products in rats can be inhibited by steroidal antiinflammatory agents suggests that, here too, lipid inflammatory compounds such as eicosanoids could act as mediators of pathologic changes induced by inhaled pollutants.

Recently, much attention has been placed on the role of metaplasia in morphologic changes leading to hypersecretion in disease states and as a consequence of toxic insults (*73,151*). One hypothesis is that the increase in numbers of mucin-secreting cells in disease or following toxicant inhalation is an adaptive response to airway injury largely involving differentiation of nonsecreting cells. Evidence supporting this notion is based on histochemical findings of increased goblet cell populations in areas of the lung where they are normally rare and in the absence of changes in the mitotic rate of cells in the airway. Preliminary work also indicates that exposure of rats to SO_2 can result in mucin gene expression changes that presumably would be required for nonsecreting cells to transform into mucin-secreting cells (*73*).

As reviewed in this report, the targets of toxicants on mucus biochemistry and physiology are numerous, and it is certain that new ones will be identified as a result of ongoing research. The significance of these alterations of mucus biology to human health is less clear, as the effect of environmentally relevant concentrations of pollutants on acute and chronic mucus hypersecretion needs to be determined. A prominent exception is cigarette smoke, for which ample epidemiologic evidence demonstrating the relationship between tobacco smoke-induced alterations in airway morphology, mucus hypersecretion and chronic bronchitis already exists. In addition, a 5-year epidemiologic study examining respiratory effects in nonsmokers found a strong correlation between persistent cough and phlegm production and long-term residence in an area with high ambient SO_2 levels (*178*). In view of these findings, it seems particularly important to determine whether pollutant-induced mucus hypersecretion contributes to the alarming rise in the number of asthma deaths that has been reported in recent years (*120*).

Many other important issues surrounding the role of mucus in the pulmonary toxicology of inhaled pollutants remain unresolved. A few are listed below.

One basic question involves whether mucin gene expression can truly be induced by toxicant exposure. If so, is this induction part of a metaplastic response or independent from it? What are the effects of toxicants on the post-translational modification of the mucin peptide, such as glycosylation and sulfation steps? Both the qualitative and quantitative changes need to be documented.

Our understanding of mucus biophysics also needs improvement. What is the true composition and function of periciliary fluid? What are the roles of other mucus components, such as surfactants and proteoglycans, in the maintenance of the rheological properties of mucus under normal and pathological conditions?

The role of inflammatory mediators in the generation and progression of inflammatory processes in the lung is one of the most exciting and promising areas of investigation in lung biology and pulmonary medicine. What mediators are involved in mucus hypersecretion induced by toxicant inhalation?

For practical reasons most experimental exposures to inhaled pollutants are acute and at high dose. What are the effects of chronic exposures to the more relevant low doses? Similarly, what are the effects of

exposure to low levels of multiple pollutants?

In summary, essentially all aspects of lung pathophysiology involving toxicant-induced alterations in mucus structure and function can be viewed in the context of the role of mucus in a stereotypical, generalized response of the lung to cellular injury. Based on our current understanding of lung physiology, it is clear that the lung can secrete mucus in response to perceived environmental challenges such as irritation of the airway mucosa. This response can be described as graded in that it seems to be proportional to the magnitude of the stimulus and its duration. Thus a minor or brief irritant exposure may produce a localized and transient discharge of mucus from existing stores in a section of the lung, while a stronger or chronic irritation may result in a full-blown response involving a long-lasting or permanent increase in the secretory capacity of the lung as a whole. One teleologic explanation of this response is that it is an effort by the lung to neutralize and remove the offending stimulus to minimize tissue injury. In this light, it is somewhat ironic that this protective mechanism can itself be both a target of inhaled pollutants and a potential source of injury to the lung. From a pulmonary health point of view, the distinction between injury resulting from effects of the toxicant on mucus biochemistry and physiology and that resulting from the adaptive secretory response to the toxicant may well be academic.

REFERENCES

1. Silberberg A. Models of mucus structure. In: Methods in Bronchial Mucology (Braga PC, Allegra L, eds). New York: Raven Press, 1988; 51–61.
2. Thornton DJ, Davies JR, Krayenbrink M, Richardson PS, Sheehan JK, Carlstedt I. Mucus glycoproteins from "normal" human tracheobronchial secretions. Biochem J 265:179–186 (1990).
3. Phipps RJ. Production of airway secretions. Semin Respir Med 5:314–318 (1984).
4. Mathews LM, Spector S, Lemm J, Plotter JL. Studies on pulmonary secretions. Am Rev Respir Dis 88:199–204 (1963).
5. Witas H, Sarosiek J, Aonu M, Murty VLN, Slomiany A, Slomiany BL. Lipids associated with rat small-intestinal mucus glycoprotein. Carbohydr Res 120:67–76 (1983).
6. Slomiany A, Liau YH, Takagi A, Laszewics W, Slomiany BL. Characterization of mucus glycoprotein fatty acyltransferase from gastric mucosa. J Biol Chem 259:13304–13308 (1984).
7. Hanson GC, Sheehan JK, Carlstedt I. Only trace amounts of fatty acids are found in pure mucus glycoproteins. Arch Biochem Biophys 266:197–200 (1988).
8. Hatch GE. Comparative biochemistry of airway lining fluid. In: Comparative Biology of the Normal Lung, Vol 1 (Parent RA, ed). Boca Raton, FL:CRC Press, 1992.
9. Welsh MJ. Abnormal chloride and sodium channel function in cystic fibrosis airway epithelia. In: The Lung: Scientific Foundations (Crystal RG, West JB, eds). New York:Raven Press, 1991; 2073–2081.
10. Mautone AJ, Cataletto MB. Mechanical defense, mechanisms of the lung. In: Pulmonary Physiology (Scarpelli EM, ed). Philadelphia:Lea and Febiger, 1990; 192–214.
11. Sheehan JK, Thornton DJ, Somerville M, Carlstedt, I. Mucin structure. The structure and heterogeneity of mucin glycoproteins. Am Rev Respir Dis 144:S4–S9 (1991).
12. Rose MC, Lynn WS, Kaufman B. Resolution of the major components of human lung mucosal gel and their capabilities for reaggregation and gel formation. Biochemistry 18:4030–4037 (1979).
13. Braga PC, Allegra L, eds. Methods in Bronchial Mucology. New York:Raven Press, 1988.
14. Braga PC, Allegra L, King M. Mathematical analysis of dynamic measures. In: Methods in Bronchial Mucology (Braga PC, Allegra L, eds). New York:Raven Press, 1988; 85–90.
15. Clarke SW. Two-phase gas-liquid flow. In: Methods in Bronchial Mucology (Braga PC, Allegra L, eds). New York:Raven Press, 1988; 125–134.
16. King M. Magnetic microrheometer. In: Methods in Bronchial Mucology (Braga PC, Allegra L, eds). New York:Raven Press, 1988; 73–84.
17. Braga PC. Sinusoidal oscillation methods. In: Methods in Bronchial Mucology (Braga PC, Allegra L, eds). New York:Raven Press, 1988; 63–72.
18. Jones R. Modification of mucus in animal models of disease. In: Proceedings of International Symposium on Mucus in Health and Disease (Elsten M, Parke DV, eds). New York:Plenum Press, 1977; 397.
19. Abraham WM. Effects of inhaled materials on airway secretions. Atmospheric pollutants and cigarette smoke. Semin Respir Med 5:324–328 (1984).
20. Roussel P, Lamblin G, Lhermitte M, Houdret N, Lafitte JJ, Perini JM, Klein A, Scharfman A. The complexity of mucins. Biochimie 70:1471–1478 (1988).
21. Jentoft N, Shogren RL, Jamieson AM, Blackwell J, Jentoft JE. Gel filtration of pig submaxillary mucin. In: Proceedings of the VIIth International Symposium of Glycoconjugates, Houston, TX 1:55 (1985).
22. Lamblin G, Lhermitte M, Klein A, Houdret N, Scharfman A, Ramphal R, Roussel P. The carbohydrate diversity of human respiratory mucins: a protection of the underlying mucosa? Am Rev Respir Dis 144:S19–S24 (1991).
23. Carlstedt I, Lindgren H, Sheehan JK, Ulmsten U, Wingerup L. Isolation and characterization of human cervical-mucus glycoproteins. Biochem J 211:13–22 (1983).
24. Boat TF, Cheng PW, Iyer RN, Carlson DM, Polony I. Human respiratory tract secretions: mucous glycoproteins of nonpurulent tracheobronchial secretions and sputum of patients with bronchitis and cystic fibrosis. Arch Biochem Biophys 177:95–104 (1976).
25. Leigh MW, Cheng PW, Boat TF. Developmental changes of ferret tracheal mucin composition and biosynthesis. Biochemistry 28:9440–9446 (1989).
26. Holden KG, Kim NCF, Griggs LJ, Weisbach JA. Gel electrophoresis of mucous glycoproteins. I. Effect of gel porosity. Biochemistry 10:3105–3109 (1971).
27. Holden KG, Kim NCF, Griggs LJ, Weisbach JA. Gel electrophoresis of mucous glycoproteins. II. Effect of physical deaggregation and disulfide-bond cleavage. Biochemistry 10:3110–3113 (1971).
28. Creeth JM. Constituents of mucus and their separation. Brit Med Bull 34:17–24 (1978).
29. Jenssen AO, Harbitz O, Smidsrod O. Electron microscopy of mucin from sputum in chronic obstructive bronchitis. Eur J Respir Dis 61:71–76 (1980).
30. Rose MC, Voter WA, Brown CF, Kaufman B. Structural features of human tracheobronchial mucus glycoprotein. Biochem J 222:371–377 (1984).
31. Sheehan JK, Oates K, Carlstedt I. Electron microscopy of cervical, gastric and bronchial mucus glycoproteins. Biochem J 239:147–153 (1986).
32. Mikkelsen A, Stokke BT, Christensen BE, Elgasaeter A. Flexibility and length of human bronchial mucin studied using low-shear viscometry, birefringence relaxation analysis and electron microscopy. Biopolymers 24:1683–1704 (1985).
33. Porchet N, Dufosse JJP, Guyonnet Duperat V, Perini JM, Nguyen V C, Degand P, Aubert JP. Structural features of the core proteins of human airway mucins ascertained by cDNA cloning. Am Rev Respir Dis 144:S15–S18 (1991).
34. Gum JR, Jr. Mucin genes and proteins they encode: structure diversity and regulation. Am J Respir Cell Mol Biol 7:557–564 (1992).
35. Carlson DM. Structure and immunochemical properties of oligosaccharides isolated from pig submaxillary mucins. J Biol Chem 243:616621 (1968).

36. Roussel P, Lamblin G, Degand P, Walker-Nasir E, Jeanloz RW. Heterogeneity of the carbohydrate chains of sulfated bronchial glycoproteins isolated from a patient suffering from cystic fibrosis. J Biol Chem 250:2214–2222 (1975).
37. Lamblin G, Rahmoune H, Wieruszeski J, Lhermitte M, Strecker G, Roussel P. Structure of two sulfated oligosaccharides from respiratory mucins of a patient suffering from cystic fibrosis. Biochem J 275:199–206 (1991).
38. Mawhinney TP, Adelstein E, Morris DA, Mawhinney AM, Barbero GJ. Stucture determination of five sulfated oligosaccharides derived from tracheobronchial mucus glycoproteins. J Biol Chem 262:2994–3001 (1987).
39. Klein A, Lamblin G, Lhermitte M, Roussel P, Breg J, Van Halbeek H, Vliegenthart JF. Primary structure of neutral oligosaccharides derived from respiratory-mucus glycoproteins of a patient suffering from bronchiectasis, determined by combination of 500 MHz 1HNMR spectroscopy and quantitative sugar analysis. Eur J Biochem 171:631–642 (1988).
40. Breg J, Van Halbeek H, Vliegenthart JFG, Klein A, Lamblin G, Roussel P. Primary structure of neutral oligosaccharides derived from respiratory-mucus glycoproteins of a patient suffering from bronchiectasis, determined by combination of 500 MHz 1HNMR spectroscopy and quantitative sugar analysis. Eur J Biochem 171:643–654 (1988).
41. Van Halbeek H, Breg J, Vlieghenthart JFG, Klein A, Lamblin G, Roussel P. Isolation and structural characterization of low-molecular-mass monosialyl oligosaccharides derived from respiratory mucus glycoproteins of a patient suffering from bronchiectasis. Eur J Biochem 177:443–460 (1988).
42. Wheater PR, Burkitt HG, Daniels VG. Functional Histology. New York:Churchill Livingston, 1979.
43. Fawcett DW. A Textbook of Histology. Philadelphia:Saunders, 1986.
44. Sleigh MA, Blake JR, Liron N. The propulsion of mucus by cilia. Am Rev Respir Dis 137:726–741 (1988).
45. Forrest JB, Lee RMKW. The bronchial wall: integrated form and function. In: The lung: scientific foundations. New York:Raven Press, 1991; 729–740.
46. Aubert JP, Porchet N, Crepin M, Duterque-Coquillaud M, Vergnes G, Mazzuca M, Debuire B, Petitprez D, Degand P. Evidence for different human tracheobronchial mucin peptides deduced from nucleotide cDNA sequences. Am J Respir Cell Mol Biol 5:178–185 (1991).
47. Jeffery PK, Reid L. Ultrastructure of the airway epithelium and submucosal gland during development. In: Lung biology in health and disease: development of the lung, vol 6 (Hodson WA, ed). New York:Marcel Dekker, 1977.
48. Jeffery PK. Morphology of the airway wall in asthma and in chronic obstructive pulmonary disease. Am Rev Respir Dis 143:1152–1158 (1991).
49. Wu R, Carlson DM. Structure and synthesis of mucin. In: The lung: scientific foundations. New York:Raven Press, 1991; 183.
50. Verdugo P. Hydration kinetics of exocytosed mucins in cultured secretory cells of the rabbit trachea: a new model. Ciba Found Symp 109:212–225 (1984).
51. Iravani J, van As A. Mucus transport in the tracheobronchial of normal and bronchitic rats. Pathology 106:81–93 (1972).
52. Toremalm NG. The daily amount of tracheobronchial secretions in a man. A method for continuous tracheal aspiration in laryngectomized and tracheotomized patients. Acta Otolaryngol 158:43–53 (1960).
53. Casarett LJ. Physical and physiological factors controlling the fate of inhaled substances. II. Retention. Health Phys 2:379 (1960).
54. Widdicombe JH. Fluid transport across airway epithelia. Ciba Found Symp 109:109–120 (1984).
55. Nadel JA, Davis B, Phipps RJ. Control of airway secretion and ion transport in the airways. Annu Rev Physiol 41:369–381 (1979).
56. Sleigh MA. Mucus propulsion. In: The lung: scientific foundations (Crystal RG, West JB, eds). New York:Raven Press, 1991; 189–196.
57. Yoneda K. Mucus blanket of rat bronchus: an ultrastuctural study. Am Rev Respir Dis 114:837–842 (1976).
58. Man SFP, Adams GK, III, Proctor DF. Effects of temperature, relative humidity, and mode of breathing on canine airway secretions. J Appl Physiol 46:205–212 (1979).
59. Rhodin JAG. Ultrastructure and function of the human tracheal mucosa. Am Rev Respir Dis 93:1–15 (1966).
60. Gilboa A, Silberberg A. In situ rheological characterization of epithelial mucus. Biorheology 13:59–65 (1976).
61. Sleigh MA. The nature and function of respiratory tract cilia. In: Lung biology in health and disease: respiratory defense mechanisms, vol 5 (Brain JD, Proctor DF, Reid LM, eds). New York:Marcel Dekker,1977; 277–288.
62. Sanderson MJ, Dirksen ER. Mechanosensitivity and beta-adrenergic control for the ciliary beat frequency of mammalian respiratory tract cells in culture. Am Rev Respir Dis 139:432–440 (1989).
63. Lansley AB, Sanderson MJ, Dirksen ER. Control of the beat cycle of respiratory tract cilia by Ca^{2+} and cAMP. Am J Physiol 263:L232–L242 (1992).
64. Wong LB, Yeates DB. Luminal purinergic regulatory mechanisms of tracheal ciliary beat frequency. Am J Respir Cell Mol Biol 7:447–454 (1992).
65. Schlesinger RB. The interaction of inhaled toxicants with respiratory tract clearance mechanisms. Crit Rev Toxicol 20:257–286 (1990).
66. Proctor DF. Form and function of the upper airways and larynx. In: Handbook of physiology. The respiratory system. Mechanics of breathing, vol 3 (Macklem PT, Mead J, eds). Bethesda:American Physiological Society, 1986; 63–88.
67. Nguyen VC, Aubert JP, Gross MS, Porchet N, Degand P, Frezal J. Assignment of human tracheobronchial mucin gene(s) to 11p15 and a tracheobronchial mucin-related sequence to chromosome 13. Hum Genet 86:167–172 (1990).
68. Gerard C, Eddy RL, Shaws TB. The core polypeptide of cystic fibrosis tracheal mucin contains a tandem repeat structure. J Clin Invest 86:1921–1927 (1990).
69. Rose MC, Kaufman B, Martin BM. Proteolytic fragmentation and peptide mapping of human carboxyamidomethylated tracheobronchial mucin. J Biol Chem 264:8193–8199 (1989).
70. Shankar V, Tan S, Gilmore MS, Sachdev GP. Molecular cloning of the carboxy terminus of a carnine tracheobronchial mucin. Biochem Biophys Res Commun 189:958–964 (1992).
71. Crepin M, Porchet N, Aubert JP. Diversity of the peptide moiety of human airway mucins. Biorheology 27:471–484 (1990).
72. Perini JM, Vandamme-Cubadda N, Aubert JP, Porchet N, Mazzuca M, Lamblin G, Herscovics A, Roussel P. Multiple apomucin translation products from human respiratory mucosa mRNA. Eur J Biochem 196:321–328 (1991).
73. Jany B, Basbaum CB. Modification of mucin gene expression in airway disease. Am Rev Respir Dis 144:S38–S41 (1991).
74. Hounzell EF, Feizi T. Gastrointestinal mucins. Structures and antigenicities of their carbohydrate chains in health and disease. Med Biol 60:227–232 (1982)
75. Boat TF, Cheng PW, Leigh M. Biochemistry of airway mucus. In: Lung biology in health and disease. New York:Marcel Dekker, 1993.
76. Loveless RW, Griffiths S, Fryer PR, Blauth C, Feizi T. Immunoelectron microscopic studies reveal differences in distribution of sialo-oligosaccharide receptors for mycoplasma pneumoniae on the epithelium of human and hamster bronchi. Infect Immun 60:4015–4023 (1992).
77. Abeijon C, Hirschberg CB. Subcellular site of synthesis of the N-acetylgalactosamine (α1-O)serine (threonine) linkage in rat liver. J Biol Chem 262:4153–4159 (1987).
78. Roth J. Cytochemical localization of terminal *N*-acetyl-D-galactosamine residues in cellular compartments of intestinal goblet cells: implications for the topology of O-glycosylation. J Cell Biol 98:399–406 (1984).
79. Phelps CF. Biosynthesis of mucus glycoprotein. Br Med Bull 34:43–48 (1978).
80. Spicer SS, Martinez JR. Mucin biosynthesis and secretion in the respiratory tract. Environ Health Perspect 55:193–204 (1984).
81. Hirschberg CB, Snider MD. Topography of glycosylation in the rough endoplasmic reticulum and golgi apparatus. Annu Rev Biochem 56:63–87 (1987).
82. Schachter H, Brochausen I. The biosynthesis of serine (threonine)-

N-acetylgalactosamine-linked carbohydrate moieties. In: Glycoconjugates; composition, structure, and function (Allen HJ, Kisailus EC, eds). New York:Marcel Dekker, 1992; 263–332.
83. Barasch J, Kiss B, Prina A, Saiman L, Gruenert D, Al-Awqati S. Defective acidification of intracellular organelles in cystic fibrosis. Nature 352:70–73 (1991).
84. Elhammer A, Kornfeld S. Purification and characterization of UDP-N-acetylgalactosamine: polypeptide N-acetylgalactosaminyltransferase from bovine colostrum and murine lymphoma BW5147 cells. J Biol Chem 261:5249–5255 (1986)
85. Shears BT, Carlson DM. Characterization of UDP-Gal:GlcNAcβ3 galactosyltransferase from pig trachea. J Biol Chem 258:9893–9898 (1983).
86. Shears BI, Carlson DM. Two distinct UDP-Gal:GlcNAc β4 galactosyltransferase in swine trachea. J Biol Chem 259:8045–8047 (1984).
87. Cheng PW, Bona S. Mucin biosynthesis: characterization of UDP-galactose:α-N-acetylgalactosamine β3 galactosyltransferase from human tracheal epithelium. J Biol Chem 257:6251–6258 (1982).
88. Brockhausen I, Moller G, Merz G, Aderman K, Paulsen H. Control of mucin synthesis: the peptide portion of synthetic O-glycopeptide substrates influences the activity of O-glycan core 1 UDPgalactose: N-acetyl-α-galactosaminyl-R β3-galactosyltransferase. Biochemistry 29:10206–10212 (1990).
89. Cheng PW, Wingert WE, Little MR, Wei R. Mucin biosynthesis: properties of a bovine tracheal mucin β6-N-acetylglucosaminyltransferase. Biochem J 227:405–412 (1985).
90. Ropp PA, Little MR, Cheng PW. Mucin biosynthesis: purification and characterization of a mucin β 6N-acetylglucosaminyltransferase. J Biol Chem 266:23863–23871 (1991).
91. Cheng PW, Moller SL, Boat TF. Properties of sialyltransferase(s) in human tracheal epithelium. Fed Proc 39:2002 (1980).
92. Schyzer M, Hill RL. Porcine A blood group-specific N-acetylgalactosaminyltransferase: 1. Purification from porcine submaxillary glands. J Biol Chem 252:2338–2345 (1977).
93. Nagai M, Dave W, Muensch H, Yoshidi A. Human blood group glycosyltransferase: II. Purification of galactosyltransferase. J Biol Chem 253:380–381 (1978).
94. Cheng PW, DeVries A. Mucin biosynthesis: enzymatic properties of human tracheal epithelial GDP-Fuc: β-galactosidase α1-2 Fucosyltransferase. Carbohydr Res 149:253–261 (1986)
95. Renosto F, Martin RL, Segel IH. ATP sulfurylase from penicilium chrysogenum. Molecular basis of the sigmoidal velocity curves induced by sulfhydryl group modification. J Biol Chem 262:16279–16288 (1987).
96. Renosto F, Seubert PA, Segel IH. Adenosine 5'-phosphosulfate from penicilium chrysogenum. Purification and kinetic characterization. J Biol Chem 259:2113–2123 (1984).
97. Verdugo P. Mucin exocytosis. Am Rev Respir Dis 144:S33–S37 (1991).
98. Fernandez JM, Villalon M, Verdugo P. Reversible condensation of mast cell secretory products in vitro. Biophys J 59:1022–1027 (1991).
99. Gallagher JT, Kent PW, Passatore M, Phipps RJ, Richardson PS. The composition of Tracheal Mucus and the nervous control of its secretion in the cat. Proc R Soc London, B, 192:49–76 (1975).
100. Widdicombe JG. Control of secretion of tracheobronchial mucus. Med Bull 34:57–61 (1978).
101. Parke DV. Pharmacology of mucus. Bri Med Bull 34:89–94 (1978).
102. McManus LM, Deavers SI. Platelet activating factor in pulmonary pathobiology. Clin Chest Med 10:107–118 (1989).
103. Kim KC. Biochemistry and pharmacology of mucin-like glycoproteins produced by cultured airway epithelial cells. Exp Lung Res 17:533–545 (1991).
104. Nadel JA. Role of mast cell and neutrophil proteases in airway secretions. Am Rev Respir Dis 144:S48–S51 (1991).
105. Lundgren JD, Shelhammer JH. Pathogenesis of airway mucus hypersecretion. J Allergy Clin Immunol 85:399–417 (1990).
106. Robbins SL, Angell M, Kumar V. Basic Pathology. Philadelphia:Saunders, 1981.
107. Lundgren JD, Kaliner M, Shelhamer JH. Mechanisms by which glucocorticosteroids inhibits secretion of mucus in asthmatic airways. Am Rev Respir Dis 141:S52–S58 (1990).
108. Wanner A. Chronic bronchitis. Sem Respir Med 5:329–331 (1984).
109. Lopez-Vidriero MT, Reid L. Bronchial mucus in health and disease. Br Med Bull 34:63–74 (1978).
110. Konstan MW, Berger M. Infection and inflammation of the lung in cystic fibrosis. In: Cystic Fibrosis (Davis P, ed). Lung Biology in Health and Disease Series, Vol 64 (Lenfant C, ed). Marcel Dekker:New York (1993).
111. Cheng PW, Boat TF, Cranfill K, Yankaskas JR, Boucher RC. Increased sulfation of glycoconjugates by cultured nasal epithelial cells from patients with cystic fibrosis. J Clin Invest 84:68–72 (1989).
112. Boucher RC, Stutts MJ, Knowles MR, Cantley L, Gatzy JT. Na+ transport in cystic fibrosis respiratory epithelia. Abnormal rate and response to adenylate cyclase activation. J Clin Invest 78:1245–1252 (1986).
113. Davis PB. Molecular and cell biology of cystic fibrosis. J Appl Physiol 70:2331–2333 (1991).
114. Rommens JH, Ianuzzi MC, Kerem BS, Drumm ML, Meimer G, Dean M, Rozmahel R, Cole JL, Kennedy D, Hidaka N, Zsiga M, Buchwald M, Riordan JR, Tsui LC, Collins FS. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 245:1059–1065 (1989).
115. Riordan J, Rommens JM, Kerem BS, Alon N, Rozmachel R, Grzetczack Z, Zielenski J, Lak S, Plavsik N, Chou JL, Drumm ML, Ianuzzi MC, Collins FS, Tsui LC. Identification of the cystic fibrosis gene: cloning and characterization of the complementary DNA. Science 245:1066–1073 (1989).
116. Kerem BS, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Muchwald M, Tsui LC. Identification of the cystic fibrosis gene: genetic analysis. Science 245:1073–1080 (1989).
117. Cheng SH, Gregory RJ, Marshall J, Paul S, Souza DW, Cohite GA, O'Riodan CR, Smith AE. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell 63:827–834 (1990).
118. Denning GW, Anderson MP, Amara JF, Marshall AJ, Smith AE, Welsh MJ. Processing of CFTR (F508) is temperature-sensitive. Nature 358:761–764 (1992).
119. Kartner N, Hanrahan JW, Jensen TJ, Naismith AL, Sun SZ, Ackerlly CA, Reyes EF, Taui LC, Rommens JM, Bear CE, Riodan JR. Expression of the cystic fibrosis gene in non-epithelial invertebrate cells produced a regulated anion conductance. Cell 64:681–691 (1991)
120. Whitelaw WA. Asthma deaths. Chest 99:1507–1509 (1991).
121. Anderson WM. Mucoid impaction of upper lobe bronchi in the absence of proximal bronchiectasis. Chest 98:1023–1027 (1990).
122. Wanner A. Bronchial asthma. Sem Respir Med 5:332–335 (1984).
123. Bateman JRM, Pavia D, Sheahan NF, Agnew JE, Clarke SW. Impaired tracheobronchial clearance in patients with mild stable asthma. Thorax 38:463–467 (1983).
124. Pavia D, Bateman JRM, Sheahan NF, Agnew JE, Clarke SW. Tracheobronchial mucociliaryclearance in asthma: impairment during remission. Thorax 40:171–175 (1985).
125. Aitken AL, Burke W, McDonald G, Shak S, Montgomery AB, Smith A. Recombinant human DNase inhalation in normal subject and patients with cystic fibrosis: a phase 1 study. JAMA 267:1947–1951 (1992).
126. Glynn A, Michaels L. Bronchial biopsy in chronic bronchitis and asthma. Thorax 15:142–153 (1960).
127. Lundgren JD, Kaliner M, Logun C, Shelhamer J. Dexamethasone reduces rat tracheal goblet cell hyperplasia produced by human neutrophil products. Exp Lung Res 14:853–863 (1988).
128. Gordon T, Amdur MO. Responses of the respiratory system to toxic agents. In: Toxicology: The Basic Science of Poisons, 4th ed Amdur MO, Doull J, Klaassen CD, eds). New York:Pergamon, 1991; 383–406.
129. Mauderly JL, Samet JM. General environment. In: The Lung: Scientific Foundations (Crystal R, West JB, eds). New York:Raven Press, 1991; 1947–1960.
130. Cross C, Halliwell B. Biological consequences of general environmental contaminants. In: The Lung: Scientific Foundations (Crystal RG, West JB, eds). New York:Raven Press, 1991;

1961–1974.
131. Graham JA. Review, discussion, and summary: toxicology. Environ Health Perspect 79:191–194 (1989).
132. Koenig JQ, Covert DS, Pierson WE. Effects of inhalation of acidic compounds on pulmonary function in allergic adolescent subjects. Environ Health Perspect 79:173–178 (1989).
133. Watanabe S, Frank R, Yokoyama E. Acute effects of ozone on lungs of cats. Am Rev Respir Dis 108:1141–1151 (1973).
134. Boucher RC. Mechanisms of pollutant-induced airways toxicity. Clin Chest Med 2:377–392 (1981).
135. Grose EC, Gardner DE, Miller FJ. Response of ciliated epithelium to ozone and sulfuric acid. Environ Res 22:377–385 (1980).
136. Dalhamn T, Sjoholm J. Studies of SO_2, NO_2 and NH_3: effect on ciliary activity in rabbit trachea of single in vitro exposure and resorption in rabbit nasal cavity. Acta Physiol Scand 58:287–292 (1963).
137. Adalis D, Gardner DE, Miller FJ, Coffin DL. Toxic effects of cadmium on ciliary activity using a tracheal ring model system. Environ Res 13:111–117 (1977).
138. Pedersen M. Ciliary activity and pollution. Lung (Suppl) 368–376 (1990).
139. Kensler GJ, Battista SP. Components of cigarette smoke with ciliary depressant activity. N Engl J Med 269:1161–1168 (1963).
140. Morgan KT, Patterson DL, Gross EA. Frog palate mucociliary apparatus: structure, function, and response to formaldehyde gas. Fundam Appl Toxicol 4:58–66 (1984).
141. Ranga V, Kleinerman J. A quantitative study of ciliary injury in the small airways of mice: the effects of nitrogen dioxide. Exp Lung Res 2:49–56 (1981).
142. Abraham WM, Sielczak MW, Delehunt JC, Marchette B, Wanner A. Impairment of tracheal mucociliary clearance but not ciliary beat frequency by a combination of low level ozone and sulfur dioxide in sheep. Eur J Respir Dis 68:114–120 (1986).
143. Battista SP, Denine EP, Kensler CJ. Restoration of tracheal mucosa and ciliary particle transport activity after mechanical denudation in the chicken. Toxicol Appl Pharmacol 22:59–69 (1972).
144. Holma B. Effects of acid on airway mucus and its consequences for health. Environ Health Perspect 79:109–113 (1989).
145. Auerbach C, Moutschen-Dahmen M, Moutschen J. Genetic and cytogenetical effects of formaldehyde and related compounds. Mut Res 39:317–362 (1977).
146. Morgan KT, Patterson DL, Gross EA. Response of the mucociliary apparatus of F-344 rats to formaldehyde gas. Toxicol Appl Pharmacol 82:1–9 (1986).
147. Last JA. Mucus production and the ciliary escalator. In: Mechanisms in Respiratory Toxicology, Vol 1 (Witschi H, Nettesheim P, eds). Boca Raton, FL:CRC Press, 1982; 247.
148. Jones R, Reid L. Secretory cell hyperplasia and modification of intracellular glycoprotein in rat airways induced by short periods of exposure to tobacco smoke, and the effect of the antiinflammatory agent phenylmethyloxadiazole. Lab Invest 39:41–49 (1978).
149. Baker AP, Sawyer JL. Glycosyltransferases in human respiratory tissue: alterations in subjects with hypersecretion of mucus. Biochem Med 14:42–50 (1975).
150. Baker AP, Chakrin LW, Sawyer JL, Munro JR, Hillegass LM, Giannone E. Glycosyltransferases in canine respiratory tissue: alterations in an experimentally induced hypersecretory state. Biochem Med 10:387–399 (1974).
151. Hotchkiss JA, Harkema JR, Henderson RF. Effect of cumulative ozone exposure on ozone-induced nasal epithelial cells and secretory metaplasia in rats. Exp Lung Res 15:589–600 (1991).
152. Harkema JR, Hotchkiss JA. In vivo effects of endotoxin on nasal epithelial mucosubstances: quantitative histochemistry. Exp Lung Res 17:743–761 (1991).
153. Phipps RJ, Denas SM, Sielczak MW, Wanner A. Effects of 0.5 ppm ozone on glycoprotein secretion, ion and water fluxes in sheep trachea. J Appl Physiol 60:918–927 (1986).
154. Stutts MJ, Bromberg PA. Effects of ozone on airway epithelial permeability and ion transport. Toxicol Lett 35:315–319 (1987).
155. Kehrl HR, Vincent LM, Kowalsky RJ, Horstman DH, O'Neil JJ, McCartney WH, Bromberg PA. Ozone exposure increases respiratory epithelial permeability in humans. Am Rev Respir Dis 135:1124–1128 (1987).
156. Foster, WM, Costa, DL, Langenback, EG. Ozone exposure alters tracheobronchial mucociliary function in humans. J Appl Physiol 63:996–1002. (1987).
157. Kim KC, Nassiri J, Brody J S. Mechanisms of airway goblet cells mucin release: studies with cultured tracheal surface epithelial cells. Am J Respir Cell Mol Biol 1:137–143 (1989).
158. McBride RK, Oberdoerster G, Marin MG. Effects of ozone on the cholinergic secretory responses of ferret tracheal glands. Environ Res 55:79–90 (1989).
159. Marom Z, Shelhamer JH, Bach MK, Morton DR, Kaliner M. Slow reacting substances, leukotrienes C4 and D4, increase the release of mucus from human airways in vitro. Am Rev Respir Dis 126:449–451 (1982).
160. Marom Z, Shelhamer JH, Sun F, Kaliner M. Human airway monohydroxyeicosatetraenoic acid generation and mucus release. J Clin Invest 72:122–127 (1983).
161. Coles SJ, Levine LR, Reid L. Hypersecretion of mucus glycoproteins in rat airways induced by tobacco smoke. Am J Pathol 94:459–472 (1979).
162. Mariassy, AT, Abraham, WM, Phipps, RJ, Sielczak, MW, Wanner, A. Effect of ozone on the postnatal development of lamb mucociliary apparatus. J Appl Physiol 68:2504–2510 (1990).
163. Spicer SS, Chakrin LW, Wardell JR. Effect of chronic sulfur dioxide inhalation on the carbohydrate histochemistry and histology of the canine respiratory tract. Am Rev Respir Dis 110:13–24 (1974).
164. Elmes PC, Bell D. Effects of chlorine gas on the lungs of rats with spontaneous pulmonary disease. J Pathol Bact 86:317–330 (1963).
165. Freeman A, Haydon GB. Emphysema after low-level exposure to NO_2. Arch Environ Health 8:125–131 (1964).
166. Rogers DF, Jeffery PK. Inhibition of cigarette smoke-induced airway secretory cell hyperplasia by indomethacin, dexamethasone, prednisilone, or hydrocortisone. Exp Lung Res 10:285–298 (1986).
167. Rogers DF and Jeffery PK. Inhibition by oral N-acetylcysteine of cigarette smoke-induced "bronchitis" in the rat. Exp Lung Res 10:267–283 (1986)
168. Last JA, Jennings MD, Schwartz LS, Cross CE. Glycoprotein secretion by tracheal explants cultured from rats exposed to ozone. Am Rev Respir Dis 116:695–703 (1977).
169. Lamb D, Reid L. Mitotic rates, goblet cell increase and histochemical changes in mucus in rat bronchial epithelium during exposure to sulphur dioxide. J Pathol Bact 96:97–111 (1968).
170. Nygren H, Lange S, Lonnroth I. Development of mucous cells in mouse intrapulmonary airways induced by cholera toxin, dibutyryl cyclic AMP and prostaglandin E_1. J Exp Pathol 65:549–556 (1984).
171. Christensen TG, Korthy AL, Snider GL, Hayes JA. Irreversible bronchial goblet cell metaplasia in hamsters with elastase-induced panacinar emphysema. J Clin Invest 59:397–404 (1977)
172. Koren HS, Devlin RB, Graham DE, Mann R, McGee MP, Hortsman DE, Kozumbo WJ, Becker S, House DE, McDonell WF, Bromberg PA. Ozone-induced inflammation in the lower airways of human subjects. Am Rev Respir Dis 139:407–412 (1989).
173. Smith J, Johnson DA. Human bronchial leukocyte proteinase inhibitor. Biochem J 225:463–472 (1985).
174. Weitz JI, Huang AJ, Landman SL, Nicholson SC, Silverstein SC. Elastase-mediated fibrinogenolysis by chemoattractant-stimulated neutrophils occurs in the presence of physiologic concentrations of antiproteases. J Exp Med 166:1836–1850 (1987).
175. Janoff A, Carp H, Lee DK. Cigarette smoke inhalation decreases α-1-antitrypsin activity in rat lung. Science 206:1313–1316 (1979).
176. Madden MC, Elin TE, Dailey LA, Friedman M. The effect of ozone exposure on rat alveolar macrophage arachidonic acid metabolism. Exp Lung Res 17:47–63 (1991).
177. Samet JM, Noah TL, Devlin RB, Yankaskas JR, McKinnon K, Dailey LA, Friedman M. Effect of ozone on platelet activating factor production in phorbol-differentiated HL60 cells. A human bronchial epithelial cell line (BEAS S6) and primary human bronchial epithelial cells. Am J Respir Cell Mol Biol 7:514–522 (1992).
178. Chapman RS, Calafiore DC, Hasselbad V. Prevalence of persistent cough and phlegm in young adults in relation to long-term ambient sulfur oxide exposure. Am Rev Respir Dis 132:261–267 (1985).